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Chapter 16

Shikimic acid as intermediary model for the production of drugs effective against influenza virus

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16.1 Introduction

Viruses are small infectious organism or obligate intracellular parasite found in virtually all ecosystem, numbering in millions. Within the last 30 years, the etiological mechanisms of some notorious viruses affecting humans have been described. Viruses are enclosed by structural coded protein coat along with either a DNA or RNA genome. The genetic material (DNA or RNA) of viruses contain information needed to replicate or to make number of copies of the virus. Viruses are considered as genetic mobile elements of mainly cellular origin, identified by an extended coevolution of host and virus. Specialized host cells providing complex biosynthetic and metabolic machinery of prokaryotic and eukaryotic organisms help in the propagation of viruses and a whole virus particle is known as virion.

Viral infections have the ability to spread from man to man or from other sources to man either by indirect or direct contact, particularly by means of excretal matter, contaminated articles, throat and nose secretions, and very common is droplet infection. An insect vector also spreads infections, e.g., transmission of dengue fever is by mosquito Aedes aegypti. A number of viral diseases are transmitted through milk and water, e.g., infectious hepatitis and poliomyelitis. Sometimes viruses change the function of the cell without destroying the host cell. Viruses might remain dormant for a period of time before multiplying again.
The genetic material of viruses controls the cells by forcing it to replicate. Generally, infected cells die as it cannot perform the normal function and after cell death it releases a new virus which continues to infect other cells. A number of viruses alter the cellular functions instead of killing the cells and these infected cells multiply abnormally by losing the control over normal cell division and turning into cancerous cells. A virus proliferates and infects cells causing viral infection.

For some viral infections, lifelong immunity is conferred after one attack of the disease such as smallpox, measles, and mumps, whereas in case of common cold, short-duration immunity is produced after an attack. In viral diseases, the mode of artificial immunization is similar to those of bacterial infections, for passive immunization dead or live vaccines are used to bring about active immunity.

Diagnostically, prospective and laboratory-based surveillance is of principal significance for the management and early detection of emerging or reemerging infectious diseases. Within the past years, many techniques have been developed for diagnosing viral infections and these procedures include (1) the detection of viral antigen in the lesions through fluorescent antibody techniques, (2) microscopic examination of the lesions, (3) performing serological tests during the course of infection, (4) skin tests, and (5) techniques for identification and isolation of virus.

16.1.1 Description of the various classes of viruses

Viruses that are pathogenic to human are broadly classified according to the different regions of the body mostly affected and clinical nature of the infection or disease produced (Table 16.1). A simple classification system of viruses is the following:

- **Neurotropic viruses**: These are viruses that affect the central nervous system, e.g., viruses of rabies and poliomyelitis.
- **Dermotropic viruses**: This class of viruses produces generalized infection by inducing characteristic lesions on the skin, e.g., viruses of measles, smallpox, and chickenpox.
- **Pneumotropic viruses**: These are viruses that produce characteristic symptoms of the respiratory tract, e.g., viruses of influenza and common cold.
- **Hepatotropic viruses**: This group of viruses infects the liver, e.g., viruses of infectious hepatitis.
- **Sialadenitis or salivary gland infection**: These are viruses that affect salivary gland or duct, resulting in viral infection such as mumps and flu.
- **Ocular viral infection**: These are viruses that affect the eyes, e.g., viruses of trachoma and epidemic keratoconjunctivitis.
- **Viruses producing generalized infections**: These include viruses of dengue fever and yellow fever.

16.2 Influenza and its various forms

Influenza is a contagious respiratory infection caused by an influenza virus, commonly known as the flu. The most common symptoms is high fever, coughing, sneezing, runny nose, headache, sore throat, muscle aches (myalgias), and a general feeling of illness (malaise). In early stage of infection, the symptoms of flu are similar to those of the common cold and commonly confused with common cold; however, it is a much more severe disease. In more serious conditions, influenza leads to pneumonia, which could be fatal, significantly in young children and the elderly. However, nausea and vomiting can be produced, especially in children, and mistaken by gastroenteritis or “stomach flu.” Typically, influenza is spread by the air because of coughs or sneezes and through infected birds because of their droppings. In addition, it can also be transmitted by direct contact with virus-contaminated surfaces and body fluids such as saliva, nasal secretions, feces, and blood.

16.2.1 Epidemiology

Each year, 10%—20% of the world’s population is affected by seasonal epidemic influenza. Seasonal influenza epidemics occur every year both in the Northern and the Southern hemispheres, mainly in the winter; however, in tropical countries, influenza can occur throughout the year. According to the World Health Organization, the annual pandemic leads to about 3—5 million cases of serious illness and about 250,000—500,000 deaths. About 20% of unvaccinated children are infected each year. Death occurs mostly in the young, the old, and those with other health problems. Antigenic shifts lead the emergence of a new and very different influenza virus that may cause an influenza pandemic. Pandemics are less frequent and result in larger outbreaks. In the 20th century, three influenza epidemics occurred: Spanish influenza in 1918
TABLE 16.1 Classification of viruses with notable examples.

| Virus group                                      | Illness (major signs)                                                                 | Incubation | Duration | Remarks                                                                 |
|-------------------------------------------------|---------------------------------------------------------------------------------------|------------|----------|-------------------------------------------------------------------------|
| Respiratory viral infections                     |                                                                                       |            |          |                                                                         |
| Rhinovirus                                       | Common cold, sneezing, coughing, sore throat, and mild headache                       | 12–72 h    | 2 weeks  | As soon as immune cells invades the local area it becomes inflamed     |
| Seasonal influenza                               | Acute respiratory infection, flu, severe fatigue, and body aches                       | 1–3 days   | 5–7 days | Antigenic makeup of virus gets significantly changed                     |
| Respiratory syncytial virus (RSV)                | Airway and lung infection, cough, and sneezes                                         | 2–8 days   | 1 days   | RSV is a single-stranded enveloped RNA paramyxovirus                    |
| Viral skin infections                            |                                                                                        |            |          |                                                                         |
| Molluscum contagiosum virus (MCV)                | Flesh-colored bumps                                                                   | 2–7 weeks  | 1 week to 6 months | MCV only replicates in human keratinocytes                           |
| Varicella-zoster virus (VZV)                     | Cold sores and genital herpes                                                          | 14–16 days | 10–21 days | It is a DNA virus of herpes virus group                                 |
| Herpes simplex virus-1 (HSV-1)                   | Chickenpox, fatigue, itchiness, oozing blisters, and high fever                       | 2–12 days  | 10 days  | Transmission of HSV-1 virus is mainly through oral-to-oral contact causing oral herpes or cold sores |
| Foodborne viral infections                       |                                                                                        |            |          |                                                                         |
| Hepatitis A                                      | Affects the liver, nausea, vomiting, diarrhea, and yellow skin                         | 14–28 days | 15–50 days | Transmission of virus is through fecal-oral route                       |
| Norovirus                                        | Gastroenteritis, vomiting, and diarrhea                                                | 12–42 h    | 24–60 h  | It is a small virus containing RNA surrounded by protein coat          |
| Rotavirus                                        | Stomach flu, dry mouth, and throat                                                    | 2 days     | 2–8 days | It is a nonenveloped, double-shelled wheel-like appearance viruses     |
| Sexually transmitted                             |                                                                                        |            |          |                                                                         |
| Human immunodeficiency virus (HIV)               | Attack T cells of immune system, rapid weight loss, night sweats, tiredness           | 1–4 weeks  | 9 months to 20 years or longer | Leading to acquired immune deficiency syndrome (AIDS)                  |
| Human papillomavirus (HPV)                       | Increases risk of cervical cancer, genital warts, or precancerous lesions              | 2–3 months | 2 years  | It is a DNA virus from the papilloma-virus family                      |
| Hepatitis B                                      | Inflammation of liver, vomiting, diarrhea, jaundice, abdominal pain                   | 75 days    | 30–180 days | Transmission of virus is through exposure to infectious blood or body fluids |
| Genital herpes or herpes simplex virus (HSV)     | Cold sores, myalgia, headache, malaise, urethral, and vaginal discharge                | 3–7 days   | 1 day to 3 weeks | HSV-1 and HSV-2 are implicated in orofacial and genital primary infections |
| Other viral infections                           |                                                                                        |            |          |                                                                         |
| Viral meningitis                                 | Inflammation in the lining of spinal cord and brain, headache, stiff neck, and fever  | 3–7 days   | 10 days  | The most common cause of viral meningitis is echovirus or coxsackie groups of enteroviruses |
| Epstein–Barr virus (EBV)                         | Swollen lymph nodes, fatigue, fever, and enlarged spleen                              | 1–2 months | 4–6 weeks | EBV is a human herpes virus causing acute infectious mononucleosis and related to autoimmune disease and cancer |
| West Nile virus                                  | Encephalitis or inflammation of surrounding tissues spinal cord and brain (meningitis) | 2–6 days   | 2–14 days | Transmission is mainly through infected mosquitoes                     |
16.2.2 Types of influenza viruses

On the basis of surface antigens, there are four genera of this family: types A, B, C, and thogotovirus. Influenza A and B are the predominant pathogen in seasonal influenza, while influenza virus A is most severe than influenza virus B and may cause severe influenza. Influenza type C infections are generally responsible for mild respiratory illness and could not cause epidemics. Influenza D viruses mainly affect cattle and are not found to infest on humans or cause illness in them.

16.2.2.1 Type A influenza

Type A influenza is the most dangerous and known to cause outbreaks and diseases. Wild birds represent the natural hosts for these viruses, while these viruses can infect animals, birds, pigs, and horses. Influenza A viruses are subdivided into a number of subtypes based on the composition of the hemagglutinin (HA) and neuraminidase (NA) proteins. There are 18 different hemagglutinin subtypes and 11 different neuraminidase subtypes (H1 through H18 and N1 through N11, respectively) exist so far. Type A flu virus is continuously changing and generally causes the large flu pandemics; however, influenza B viruses are not responsible for pandemics. Mutation in influenza viruses resulted in a number of different influenza subtypes and strains; it is more common in influenza A virus. Specific varieties of the virus are generally named according to the host of origin, geographical location, strain number, and year of isolation.

16.2.2.2 Influenza B

Influenza B infections occur only in humans and are not divided into subtypes, but can be further broken down into lineages and strains. Currently circulating influenza B viruses belong to one of two lineages: B/Yamagata and B/Victoria.

16.2.2.3 Influenza type C

Influenza type C infections cause a mild respiratory illness (comparable to other common respiratory viruses) and nearly all adults have been infected with influenza C virus. However, it is milder and rare than either type A or B and does not cause epidemics. The structures of influenza C viruses are different in comparison to influenza A and B. The genera C virion has hexagonal structures on the surface and forms stretched cord-like structures. Influenza C viruses have seven RNA segments, while influenza A and B contain eight RNA segments.

16.2.3 FDA-approved drugs for influenza

Influenza is a serious infectious disease, which is very dangerous and deadly specifically in young one, elderly, and weak immunity patients. After the invention of modernized vaccinations, the mortality rate due to flu virus has been considerably decreased, but there are chances of recurrent infestation. Vaccination, accompanying the emergence of new antiinfluenza agents, is necessary to fight against seasonal and pervasive influenza strains. Nowadays, most importance is given to the designing of inhibition agents of influenza neuraminidase, which has proved itself as an appropriate medicine for the prophylaxis and influenza infections therapy. Till now only four medicines have been accepted by the Food and Drug Administration (FDA) to cure influenza ailment, of which two of these drugs have grown resistance for influenza A strains and hence are not in the application. Therefore, there are a numerous chances in this area for further development of antiviral therapy. The two recent drug mechanisms for inhibition of the influenza virus are the M2 proton channel, which involves the procedure of liberating virus particles from an infected host cell, and the neuraminidase (NA) enzyme, which works on the basis of viral recognition and entry into a host cell. Currently, neuraminidase inhibitors are the most frequently used FDA-approved treatments for the influenza virus, which is applied effectively as they have not developed any resistance yet within the virus. Oseltamivir and zanamivir are the drugs that function by affecting the activity of neuraminidase (NA) protein of the virus. The neuraminidase enzyme operates on the basis of cleaving the host cell sialic acid residues with viral hemagglutinin, in turn releasing the new viruses to carry forward and infect other healthy cells. The viral neuraminidase catalytic site is highly specific for all influenza A and B viruses. Both the drugs oseltamivir and zanamivir serve as stable transformation state counterparts of the sialosyl cation. It is a very unstable transformation state complex in the enzymatic procedure of viral sialidase. The neuraminidase inhibitor drugs 1 and 2 are similar enough in...
structure to the sialosyl cation transition state complex. It allows them to attach tightly to the sialic acid active site and obstruct viral budding. However, constant development of drug-resistant influenza strain enhances the need of continuous researches on innovative technologies for the production of new drugs with improved antiviral mechanism, more safety, and enhanced tolerability.

16.2.3.1 Influenza virus inhibitors

Eight drugs have been accepted to cure influenza virus infections on April 2016. These drugs could be classified as neuraminidase inhibitors, e.g., oseltamivir, zanamivir, peramivir, and laninamivir octanoate, matrix 2 inhibitors, e.g., rimantadine and amantadine, and polymerase inhibitors such as favipiravir and ribavirin.

16.2.3.1.1 Rimantadine and amantadine

Amantadine (1-adamantanamine) approved in 1966 was the first antiviral compound for the treatment of widely distributed influenza A virus infections. This compound has the ability to block the transfer of H ions with the help of M2 (matrix 2) protein medium into the inner portion of viral particles and hence prevents the disassembling of influenza virus molecule inside the endosomes. Amantadine and rimantadine were approved for adult patients, but they were not able to prevent, treat, or reduce the severity of influenza A virus infestation in children and older ones. The prevalent resistance also leads to abandon amantadine in the medication of influenza infections. However, the interest is growing for the application of amantadine in the evidential therapy of Parkinson disease and levodopa-induced dyskinesia, although more clinical researches are necessary to explore its new application.

16.2.3.1.2 Zanamivir, oseltamivir, peramivir, and laninamivir octanoate

Zanamivir is a distinct and potent inhibitor of neuraminidases protein of influenza A and B virus. It prevents the influenza infections by affecting release of virus. Zanamivir applied by inhalation was also accompanied with oseltamivir, ingested by the oral route. According to many researches, oral oseltamivir and inhaled zanamivir have proved to be a net benefit by controlling death rate and the severity of influenza symptoms and complications. Two more neuraminidase inhibitors were introduced for the therapy of influenza infections after the success of zanamivir and oseltamivir. First was peramivir, which could be administered as an intravenous injection, and the other was laninamivir octanoate, which was found to be effective if administered in an inhalation. Notably, peramivir has been found to have clinical potency equal to that of oseltamivir for curing the severe seasonal influenza, and the efficacy of laninamivir octanoate has been found for the treatment of seasonal influenza, as well as oseltamivir-resistant virus, in adults. Both peramivir and laninamivir are effectively used as a one-dose therapy for influenza A and B viruses, but their applications are bounded in very few countries only.

16.2.3.1.3 Ribavirin

Ribavirin also known as Virazole (1-n-ribofuranosyl-1,2,4-triazole-3-carboxamide) has been reported as the first synthetic nucleoside analogue, which is active toward a broad spectrum of RNA viruses (RSV, HCV, and influenza virus). It was found to be the broadest spectrum antiviral agent ever reported for a synthetic material which did not induce interferon. The chief mechanism of its drug action includes the inhibition of inosine-5-monophosphate (IMP) dehydrogenase, which helps in conversion of IMP to xanthosine monophosphate and thus responsible for the production of GTP. Ribavirin inhibits the IMP dehydrogenase and hence possesses immunosuppressive activity, which leads to the remarkable success of ribavirin, for the therapy of HCV infestation accompanied with peginterferon alfa-2a. The patients of HCV infection undergoing therapy of telaprevir, peginterferon alfa-2a, and ribavirin are found to have an outstanding level of response against the virus. Ribavirin effectively hinders the RNA polymerase of influenza virus in the triphosphate form. Apart from this, ribavirin could also be used in the treatment of “Lassa fever” (hemorrhagic fever virus infection), but ribavirin has yet not got the formal license for this medication. The effect of ribavirin on RNA viruses has also been found to have potential effects against many other infectious diseases such as dengue virus, norovirus, Hendra and Nipah viruses, and Marburg virus. However, more clinical evidence is still necessary to prove these new applications.

16.2.3.1.4 Favipiravir

Favipiravir (T-705), having chemical structure as 6-fluoro-3-hydroxy-2-pyrazine carboxamide, has been chiefly introduced for therapy of influenza virus infections. Favipiravir was invented and approved in Japan and could be effectively used for the treatment of influenza A, B, and C virus infections. Importantly, favipiravir triphosphate is a broad-spectrum antiviral
agent and shows inhibitory activities against the RNA polymerases present in influenza A viruses. Highly pathogenic H5N1 viruses and many other positive- and negative-sense RNA viruses also belong to the group of influenza A virus and hence favipiravir is active against them. Recently, favipiravir was found effective to cure patients infected because of Ebola virus (EBOV). It also inhibits the growth of human norovirus and human arena viruses (Machoito, Junin, and Pichinde viruses), but these new applications need further extensive researches and clinical trials.

16.2.4 Plant-derived phytochemicals with antiviral effects

Plants are the foundational element of many medicinal structures under application today. About 50% of the drugs prescribed are either directly extracted from plants or are derivatives of products resulted from plants [1]. Antivirals extracted from natural origin have found to possess pharmacological and pharmaceutical properties. Using different medicinal plants in combination therapy has been proved to be effective against a numerous viruses such as HSV and influenza viruses [2,3]. Many plant extracts are observed to have broad spectrum of antiviral activity. For example, Ocimum basilicum and Agrimonia pilosa are potent against a wide range of RNA and DNA viruses [4–6]. Many phytochemicals may also have dose-dependent viral inhibition [7,8]. Moreover, they are resolving an important issue of drug resistance generated because of synthetic drugs [9], e.g., a plant-derived product. Polycitone A is functional against the resistant strains of HIV [10]. Plant-derived products are also inexpensive and could be easily accessible in different parts of the world. Natural products are even found to be less toxic, cheaper, and impart no side effects in comparison with the synthetic drugs. Apart from this, they have proved their wide therapeutic benefits for different types of conditions. Plant-derived ingredients have shown different kind of mechanisms against the activities of viruses:

16.2.4.1 Immunomodulators

The enhancement of defensive immune reaction is one of the most important mechanisms of antiviral treatment. Many of the recently registered products are working on the immunity boosting procedures toward viral infections. Interleukins, colony-stimulating factors, and interferons are the most well-known immunostimulants. Interferons, which are the derivable polypeptides and glycoproteins, act as catalyst to enhance the growth of certain peculiar enzymes that control viral reconstruction in the cell [11,12]. Interleukins are the factors that increase the activation, development, distinction, progression, and guidance of immune cells, which can be able to nullify the virus [13]. Similarly, colony-stimulating factors regulate the proliferation and distinction of progenitor cells in the white blood cells lineage [14]. However, many of the drugs, such as ribavirin, also affect positively the immune responses [15]. Many of natural materials have been researched for their immunomodulatory activities. Carbohydrates, stilbenoids, alkaloids, polyphenols, lectins, and peptides from plant sources are the chief categories of drugs that could be used as immunomodulators.

16.2.4.2 Virus attachment and entry inhibitors

Another most important target for the antiviral therapy is the adjunction of virus to the host cell and its entrance. The entry of the virus occurs into the cell by interacting either by a single cell surface receptor or by certain coreceptors. After that, the viral envelop gets fused with host cell membrane, and as it intrudes into the cell, the virus is dismantled to release its genome. Many of the approved drugs affect this procedure of viral infection, e.g., tromantadine for the treatment of HSV infections changes the glycoproteins present on the surface of the host cells and stops the adhesion, intrusion, and uncoating of the virus. Studies on different plant-derived materials have shown the similar mechanisms for preventing viral growth, e.g., plant lectins extracted from genera Hippeastrum and Galanthus have shown the inhibition activity against the HIV-specific glycoproteins, thus resulting in inhibiting the entrance of virus into the cell. Other categories of plant materials, which includes, galactose, glucose, and N-acetylgalactosamine, have been found to contain antiviral action against severe acute respiratory syndrome corona virus (SARS-coV) and the feline infectious peritonitis virus. These factors also inhibit the viral adhesion to the host cell. Many of natural agents also work against influenza virus receptor attaching and merging protein, i.e., hemagglutinin. In many studies, retardation of virus dismantling and emergence of genetic matter into the cell has been done by the extracts derived from various seaweeds. Heparin sulfate molecules extracted from carrageenans, seaweed, also have shown to employ antiviral activity toward dengue virus by arresting the uncoating of virus in host cells.

16.2.4.3 Modifiers of viral genome and protein processing

Viral arrangement and mutation processes are the next important mechanism for an antiviral policy. The genome could be managed earlier, and it can utilize the cellular machinery to its benefit (RNA viruses) or it could directly integrate to the
host genome (DNA viruses). Reverse transcription, integration, replication, transcription, and translation are the potential steps of this procedure. Coumarins also known as the calanolides are extracted chiefly from *Calophyllum lanigerum* and belong to eminent category of plant-derived antivirals. They have been found to irreversibly bind to the active site of the reverse transcriptase enzyme. Large quantity of calanolides could be extracted from the latex of relevant species of the plant. Calanolide are also chosen for phase II clinical studies for antiretroviral treatment and has shown a synergistic effect with currently approved drugs.

### 16.2.4.4 Virus assembly and release inhibitors

These medicines hinder the transcription of newly amalgamated viral proteins into virions and their liberation out of the cell. Neuraminidase inhibitors work by hampering the influenza virus liberation out of the infected cells, hence resulting in safeguarding cell-to-cell transmission. Zanamivir and oseltamivir are the two most important parts of the approved drugs of this group. Up till now, more than 30 various types of protease inhibitors are extracted from plants possessing potential antiviral activities. Compounds of different botanical and chemical sources have been studied for this purpose. Extracts from *Zingiber zerumbet*, *Orostachys japonicus*, *Boesenbergia pandurata*, *Alpinia galanga*, *Coccinia grandis*, *Cassia garretiana*, and *Eclipta prostrata* have proved their antiviral potential exploiting their protease inhibiting property. Bevirimat extract obtained from a Chinese herb, *Syzygium claviflorum*, has been found to have “maturation inhibition” capability [16]. Same as the protease inhibitors, this compound possesses the ability of suppressing the cleavage of Gag polyprotein of HIV. Hence, both the structural and enzymatic proteins are not formed. Another Chinese preparation, Ching-fang-pai-tu-san consumed as a traditional herbal decoction, contains quercetin, isoquercetin, and chaihu. These compounds stop influenza virus replication by interrupting the intracellular protein processing, transportation, and budding properties [17].

### 16.2.5 Shikimic acid and its mode of action on influenza virus

Shikimic acid having a chemical formula 3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid is an organic compound found naturally and is an important link in the pathway for the development of aromatic amino acids, lignin, and various alkaloids present in plants and microorganisms.

Shikimic acid is a primary progenitor of the pharmaceutical manufacturing as antiinfluenza drug oseltamivir. Oseltamivir is marketed under the brand name Tamiflu. It is a potential antiviral medicine used to cure and prevent influenza A and influenza B infections. The mode of application is by mouth, either in the form of pill or liquid. Oseltamivir was the first neuraminidase inhibitor available orally. It was approved for medical use and now found in the complementary list of World Health Organization’s List of Essential Medicines, indicating a lower cost–benefit ratio. Its generic version was approved in the United States in 2016.

Oseltamivir is a compound acting as neuraminidase inhibitor, which is a potential inhibitor of influenza’s neuraminidase enzyme. Neuraminidase inhibitors (NAIs) define a class of drugs that stop and block the activity of neuraminidase enzyme and hence they are generally utilized as antiviral medicines. They inhibit the action of viral neuraminidases present in influenza viruses and prevent their reproduction through budding in the host cells. This enzyme is responsible for cleaving the sialic acid, which is present in glycoproteins on the surface of human cells, which in turn helps new virions to come out from the cell. Oseltamivir inhibits the function of neuraminidase protein and prevents new viral particles from being released, so that the virus cannot leave the cell to infect other cells. Eventually, the virus dies.

#### 16.2.5.1 Plant sources of shikimic acid

The isolation of shikimic acid from the fruit of *Illicium religiosum* was primarily reported by Ekmann in 1885 [18]. The name shikimic acid was derived after the oriental plant shikimi-no-ki in Japanese. Shikimic acid is now available in more quantities through the extraction from the fruit *Illicium verum* (Chinese star anise). It was recently reported that *Liquidambar styraciflua*, more commonly known as the sweetgum tree, could be a renewable source of shikimic acid. In fact, it was shown that the sweetgum tree can yield shikimic acid in amounts comparable with that of *I. verum*, through the seeds of its annual fruit. Other plants to which shikimic acid have been isolated from were presented in Table 16.2.

#### 16.2.5.2 Biosynthesis of shikimic acid in plants

Shikimic acid, obtained from star anise present in its anionic form shikimate, is a cyclohexanecarboxylic acid, a cyclohexene, and a cyclitol. The shikimic acid is formed through the following biosynthesis pathway as described in Fig.16.1.
### TABLE 16.2 Plant sources of shikimic acid.

| Plant source       | Organ or part of plant | References                     |
|--------------------|------------------------|--------------------------------|
| Iris pseudacorus   | Rhizome                | Henshaw et al. [19]            |
| Picea pungens      | All branches           | Neish [20]                     |
| Picea glauca       | All branches           | Neish [20]                     |
| Eucalyptus sieberiana | Cambium             | Hillis [21]                    |
| Eucalyptus regnans | Cambium                | Hillis [21]                    |
| Ginkgo biloba      | Inner bark, leaves     | Hasegawa and Tateoka [22]      |
| Pinus thunbergii   | Leaves                 | Hasegawa and Tateoka [22]      |
| Liquidambar styraciflua | Seeds            | Enrich et al. [23]             |
| Pinus densiflora   | Roots, seeds, bark, leaves | Hasegawa et al. [24]         |
| Pinus thunbergii   | Roots, seeds, bark, leaves | Hasegawa et al. [24]         |
| Illicium anisatum  | Roots, seeds, bark, leaves | Hasegawa et al. [24]         |
| Magnolia grandiflora | Roots, seeds, bark, leaves | Hasegawa et al. [24]         |
| Houttuynia cordata | Roots, seeds, bark, leaves | Hasegawa et al. [24]         |
| Saxifraga stolonifera | Roots, seeds, bark, leaves | Hasegawa et al. [24]         |
| Terminalia arjuna  | Fruits                 | Bharathi et al. [25]          |
| Pistacia lentiscus  | Whole plant            | Bharathi et al. [25]          |
| Ribes aureum       | Whole plant            | Bharathi et al. [25]          |
| Symphytum officinale | Leaf                | Bharathi et al. [25]          |
| Actaea pachypoda   | Whole plant            | Bharathi et al. [25]          |
| Alangium salvifolium | Root                | Bharathi et al. [25]          |
| Veratrum viride    | Leaf                   | Bharathi et al. [25]          |
| Dipsacus laciniatus | Leaf                   | Bharathi et al. [25]          |
| Agastache urticifolia | Whole plant        | Bharathi et al. [25]          |
| Inula helenium     | Leaf                   | Bharathi et al. [25]          |
| Hypericum spp.     | Whole plant            | Bharathi et al. [25]          |
| Commelina benghalensis | Stem              | Bharathi et al. [25]          |
| Gymnema sylvestris | Leaf                   | Bharathi et al. [25]          |
| Terminalia chebula | Fruits                 | Bharathi et al. [25]          |
| Illicium floridanum | Leaf                   | Bharathi et al. [25]          |
| Illicium diffenri | Fruits                 | Bharathi et al. [25]          |
| Illicium henryi    | Fruits                 | Bharathi et al. [25]          |
| Illicium verum     | Fruits                 | Bharathi et al. [25]          |
| Illicium lanceolatum | Fruits             | Bharathi et al. [25]          |
| Illicium pachyphyllum | Fruits          | Bharathi et al. [25]          |
| Illicium anisatum  | Fruits                 | Bharathi et al. [25]          |
| Illicium religiosum | Fruits                 | Bharathi et al. [25]          |
| Hemidesmus indicus | Root                   | Bharathi et al. [25]          |
| Cistus incanus     | Whole plant            | Bharathi et al. [25]          |
| Sida acuta         | Whole plant            | Bharathi et al. [25]          |
| Celastrus paniculatus | Leaf                | Bharathi et al. [25]          |
| Glycosmis muricata | Root                   | Bharathi et al. [25]          |

*Continued*
Phosphoenolpyruvate reacts with erythrose 4-phosphate to yield 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP), in the presence of catalyst, i.e., enzyme DAHP synthase. DAHP then changes to 3-dehydroquinate (DHQ), in the presence of enzyme DHQ synthase.

DHQ is dehydrated to 3-dehydroshikimic acid by the enzyme 3-dehydroquinate dehydratase in the next biosynthetic step, which transformed into shikimic acid in the reduction reaction catalyzed by the shikimate dehydrogenase. Nicotinamide adenine dinucleotide phosphate (NADPH) contributes as a cofactor in this reduction reaction.

Shikimic acid, a natural compound, has key role as an intermediary substance in the manufacturing of amino acids. Therefore, this derivative is wide spread in various plants and has potential biological properties. Apart from this, shikimic acid has pharmacological relevance because it also acts as an intermediate in the manufacturing of many drugs and the mostly related to antiviral agent called oseltamivir (Tamiflu).

### 16.2.5.3 Other methods for the synthesis of shikimic acid

#### 16.2.5.3.1 Chemical synthesis

The most common method for synthesis of racemic SA was obtained by Diels Alder reaction with (1E, 3E)-1,4-diacetoxy-1,3-butadiene, and in this method acrylic acid was used as starting material for the SA synthesis with 15% yield [27,28].

**TABLE 16.2 Plant sources of shikimic acid.—cont’d**

| Plant source              | Organ or part of plant                        | References          |
|---------------------------|-----------------------------------------------|---------------------|
| *Tanacetum parthenium*    | Leaf and blossom                              | Bharathi et al. [25]|
| *Triticum aestivum*       | Leaf                                          | Bharathi et al. [25]|
| *Hypericum dolabriforme*  | Whole plant                                    | Bharathi et al. [25]|
| *Dipsacus pilosus*        | Leaf                                          | Bharathi et al. [25]|
| *Triadenum walteri*       | Whole plant                                    | Bharathi et al. [25]|
| *Hypericum fronosum*      | Whole plant                                    | Bharathi et al. [25]|
| *Terminalia pallid*       | Leaf                                          | Bharathi et al. [25]|
| *Hemidesmus indicus*      | Whole plant                                    | Bharathi et al. [25]|
| *Chelidonium majus*       | Whole aboveground part with blossoms           | Bochkov et al. [26] |
| *Ribes aureum*            | Blossoms and young sprouts                     | Bochkov et al. [26] |
| *Pteridium aquilinum*     | Aboveground part before expansion              | Bochkov et al. [26] |
| *Populus nigra*           | Leaves                                        | Bochkov et al. [26] |
| *Acer negundo*            | Seeds                                         | Bochkov et al. [26] |
| *Pinus sylvestris*        | Summer, winter needles                         | Bochkov et al. [26] |

![FIGURE 16.1 The shikimic acid biosynthesis pathway.](image)
Other studies have also reported that SA was synthesized from (-)-quinic acid obtained from cinchona bark [29]. Grewe and Hinrichs [30] also reported a synthesis of SA, but achieved only 11% overall yield. Koreeda and Ciufolini [31] achieved a higher yield (29% overall yield). Among all the highest yields, up to 55% was found in synthesis by Fleming.

16.2.5.3.2 Microbial synthesis
The SA first isolated of microbial source was from Escherichia coli by Millcan [32]. It was also reported that bacillus species increases the yield of SA by initiating the activity of shikimate dehydrogenase and shikimate kinase enzyme. Other strains of E. coli such as E. coli SP1.1/pKD12.112 yields 20.2 g/L culture while W3110.shik 1 yields a significant amount of SA through the increase of the activity of kinase II enzyme for the synthesis of SA.

16.2.5.3.3 Enzymatic approach
Adachi et al. [33] have introduced a novel method for the enzymatic synthesis of shikimic acid from quinic acid with the help of dried cells or the membrane portion of gluconobacter oxydans IFO 3244. The enzymes obtained quinate dehydrogenase and 3-dehydroquinate dehydratase and were utilized to transform quinic acid into 3-dehydroshikimate. Dehydroshikimate was then treated with NADPH-dependent d-glucose dehydrogenase and shikimate dehydrogenase for obtaining shikimic acid. This technique is suitable for laboratory recovery of shikimic acid only but not from the perspective of industrial production.

16.2.5.4 Usefulness of shikimic acid pathway
The shikimic acid pathway is a biosynthetic pathway which is absent in mammals. It involves the metabolism of carbohydrates and allows the biosynthesis of aromatic amino acids and aromatic compounds such as naphthoquinones, ubiquinones, and folates. The shikimate pathway is a part of the metabolism occurred only in plants and microorganisms such as microbial eukaryotes, bacteria, and parasites. The sequence of seven-step shikimate pathway consists of transforming phosphoenolpyruvate and erythrose-4-phosphate starting with the condensation of them. Their condensation and cyclization lead to the synthesis of chorismic acid. Active forms of these with coenzyme A (CoA) can lead to the formation of phenolic compounds, which in turn by β-oxidation changes to acids of the benzoic acid series (gallic, protocatechuic, etc.). Gallic acid in combination with simple sugars transforms to the tannin compounds such as gallic and ellagic tannins and, after the addition of a molecule of phosphoenolpyruvate and additional intermediate stages, leads to the formation of aromatic amino acids tyrosine and phenylalanine amino acids. The pathway includes various enzymes such as 2-keto-3-deoxy-d-arabinoheptulosonate-7-phosphate synthase, 3-enolpyruvylyshikimate-5-phosphate synthase, shikimate dehydrogenase, shikimate kinase, 5-dehydroquinate dehydratase, and chorismate synthase. Shikimic acid is the intermediate product of this biological pathway by shikimate dehydrogenase, which facilitates the reversible transformation by reduction of 3-dehydroshikimate into shikimate. The shikimic acid pathway provides a new methodology for developing new herbicides and herbicide-resistant crops. It also provides the opportunity for engineering new antibiotic and antiparasitic drugs by interrupting the action of its enzymes. Hence, various important researches are being carried out to explore this pathway.

16.2.5.5 Shikimic acid isolation from plant raw materials
The Illicium genus is the basic source of shikimic acid, and the acid was discovered in 1885 from its fruits. The techniques are constantly improving for isolation of shikimic acid from these plants’ fruits. Adams et al. [34] extracted shikimic acid of 98% purity and >5% yield based on a dried fruit weight obtained with the help of Soxhlet extractor in 24 h extraction and subsequent purification by a Solka-Floc anion exchange resin from 900 g of Illicium anisatum seeds. Adams’s method was modified and simplified by Payne and Edmonds [35] and improved the yield of shikimic acid up to 7% on a dry basis. In their method, about 25 g of I. anisatum seeds were ground to dust and were extracted in a Soxhlet extractor with 95% ethanol as a solvent for approximately 2 h. The extract was then treated with water and few drops of formaldehyde solution to obtain an orange clear solution which was passed through an anion exchange column (Amberlite IRA-400, acetate form, dry weight 25 g). Shikimic acid was than eluted with acetic acid and the yellow eluent was collected, which was again heated with methanol, activated carbon and recrystallized with toluene to yield a brilliant white, crystalline substance. The modified Adams’s and Payne’s methods are being exploited in China for the industrial production of shikimic acid from Illicium genus plants.

Weinstein et al. [36] have firstly isolated high purity C14-labeled shikimic acid from Ginkgo biloba L. and conducted experiments for studying the metabolism in plants, especially for studying the shikimate pathway. For this purpose, the plant was kept for metabolism in an atmosphere of radioactive carbon dioxide for several days. Extraction of the plant material was done with ethanol and water, and the impurities of the extract were removed by passing it through a Dowex...
50-X4 (H+ form) column. The column of Dowex 1-X8 (acetate form) was used to pass the eluate. The excellent separation of shikimic and quinic acids was achieved by gradual elution with an aqueous acetic acid, and sufficiently pure compounds were found after the final passage through the Dowex 1-X8 column. 2.12 g of shikimic acid and 0.593 g of quinic acid were yielded by 287 g of fresh leaves. G. biloba L. is known to yield shikimic acid no less than 4% based on wet leaves. During chromatographic purification, high loss of shikimic acid occurs and hence the above technique gave an extremely low yield (below 20%). A modified technique was employed by Underhill et al. [37] in which Ginkgo rose shoots were grown with each other in a radioactive atmosphere, which yielded higher content of shikimic acid (2.5% on a dry basis of Ginkgo leaves) than in case of using Ginkgo only. These techniques are important from the perspective of research but could not be used to the industrial-level production of shikimic acid as they are multistage process and results in high product loss.

An efficient method for isolating shikimic acid from leaves of plants of the Liquidambar genus was developed by Li et al. [38]. In the process, fruit coatings and leaves of the Liquidambar genus trees were dried at 65°C and milled, which was further soaked in deionized water for 4 h subsequently three times. The extracts were then passed through Amberlite IRA-400 (acetate form), and shikimic acid was eluted with 25% acetic acid, concentrated, and then crystallized from the mixed ethyl acetate and methanol. The shikimic acid was obtained of 98% purity and 70% yield depending on the original content of shikimic acid in the Liquidambar fruits leaves, which is approximately 3%–8% on a dry basis, depending on a species. The above-described method is being actively exploited in the Chinese industry for the production of shikimic acid from the Liquidambar genus plants.

16.2.5.6 Mechanism of action of oseltamivir

As discussed above, oseltamivir commercialized under the trade name Tamiflu is an antiviral drug, which is being synthesized from shikimic acid. The oseltamivir help to lower down the rate of spread of influenza (flu) virus among cells in the body by interrupting the removal of the virus from its host cell. The drug is ingested orally in capsules or in the form of suspension. Both influenza A virus and influenza B virus may be treated by it.

After ingestion of oseltamivir, firstly it acts like an inactive chemical or prodrug which changes into its active state by metabolic process inside the liver, where because of hydroxylation, it changes to active metabolite—the free carboxylate of oseltamivir (GS4071). It was the firstly used orally active neuraminidase inhibitor, which serves as a competitive inhibitor for the action of the viral neuraminidase (NA) enzyme on sialic acid, present on glycoproteins found on the surface of normal host cells. By hindering the activity of the enzyme, oseltamivir stops new viral particles from being formed by infected cells. Oseltamivir is indicated for the treatment and prevention of infections due to influenza A and B viruses. In gastrointestinal tract, there is approximately 80% bioavailability of oseltamivir phosphate, which made it readily absorbed in the cells. Consequently, the oseltamivir is well distributed after ingestion as pill to the nasal mucosa, the tracheal lining, and the tissues of the middle ear. The elimination of oseltamivir carboxylate from the body follows the route for its elimination by glomerular filtration and then renal tubular excretion without undergoing further metabolism process. The estimated average half-life of elimination in adults ranges from 6 to 10 h. Influenza A and B are the two most important viruses responsible for the yearly flu seasons in human beings. They are named on the basis of major types of antigenic proteins present on the viral coating. These antigenic proteins are hemagglutinin (HA) and neuraminidase (NA), out of which the NA is a primary target of oseltamivir and zanamivir.

16.2.6 Conclusion

Despite the rapid growth of pharmaceutical and biotechnological approaches, the development of effective antiviral therapy is still a challenge. Influenza has created a global menace for the society as it has yet not developed the appropriate technology to manage the epidemic from resistant strains. Apart from the emergence of drug resistance, development of mutant strains of the virus, introduction of a more virulent strain, expensive available drugs, time lapse in vaccine manufacture, and mass mortality cause difficult problems. In this scenario, counterpart and alternate medicine offers huge possibilities to help patients. Herbs possess a wide array of biological actions and could be efficiently exploited for managing pandemic flu. Nutritional and botanical properties together are responsible for providing potent tools for controlling different types of viral infections. The accessibility of a wide array of herbs and constituents with potentially active phytochemicals, to increase the effect as antiinfluenza agents, could have an important role in the ongoing research toward the novel H1N1 infection. Shikimic acid is also found as potential antiviral source as it could be used as a precursor for industrial production of the antiviral oseltamivir or Tamiflu, a potent viral neuraminidase inhibitor. Rigorous researches for optimization of shikimic acid production and its utilization can bring a revolutionary change in antiviral therapy.
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