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## CHAPTER 3

# Ethnomedicinal Wisdom: An Approach for Antiviral Drug Development

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### 3.1 ETHNOMEDICINE: A BOON

A famous quote of Norwegian Artist Edvard Munch “*Nature is not all that is visible to the eye. . . also includes the inner pictures of the soul*” speaks many things about the mother “Nature” and her creation. For the past 100 years, mankind have been trying to unravel the secrets of nature, yet continue to be surprised by its revelations day by day. Nature is vast in its resources which not only provide food, clothes, shelter, and antiques but also provide medicine since ages. Out of these resources the greatest gift of nature is her atmosphere with food and medicine, which not only to help mankind to sustain but also to grow, develop, and survive, particularly from the diseases and sufferings. The study of natural resources traditionally used to cure or manage ailments in diverse ethnic culture is collectively termed as “Ethnomedicine” (Chattopadhyay, 2010; Chattopadhyay et al., 2009a).

Scientifically, “Ethnomedicine” is the study of “traditional medicine” of ethnic communities, their knowledge and practices that transmitted orally over centuries, and evolved over millennia of human existence (Chattopadhyay, 2009, 2010). The indigenous people of India till date used their medicaments or “so called medicines” which might be more appropriately defined as the use of plants in the treatment of diseases and should be more accurately termed as “Ethnobotanical medicine” (Fabricant and Farnsworth, 2001). For a considerable period of time, traditional medicine and ethnomedicine were ignored by the clinicians and biomedical practitioners due to a number of factors including the

questionable purity, safety, and potency. The raw material to prepare those medicines is not standardized through modern quality control parameters; its chemical profile and their quantification are not known or maintained, and thus their purity is in question. Similarly, their toxicity profile at low doses for long term is unknown, so long-term use in tolerable dosage need to be monitored to rule out the question of long-term toxicity, if any. Although the traditional medicines are used for generations with limited or no major toxic manifestations, which can be considered as “Proof of Concept” in nature’s laboratory. In 21st century the efficacy of any therapeutically useful product should be quantified in terms of modern medicine, so the efficacy of useful traditional and ethnomedicinal plants needs to be validated in modern laboratory by establishing the dosage and the exact mode and mechanism of action in one hand and *in vivo* efficacy in suitable animal models on the other. Moreover, if possible use of *ex vivo* model than can mimic the human body for better and global acceptability. With the upsurge research, ethnomedicinally useful plants have become one of the most acceptable resources not only for the pharmaceutical industry to develop therapeutically useful leads, or pharmaceuticals, but also to help in developing supplements or nutraceuticals, and even cosmetics. Reports from WHO-Traditional Medicinal Programme showed that a total of 122 compounds were isolated from 94 plant species, 80% of which were used for the same or related ethnomedical purposes (Fabricant and Farnsworth, 2001). Since these compounds were derived only from 94 species of plants out of an estimated 250,000 flowering plants, one might imagine the abundance of drugs remaining to be identified from plant kingdom.

### 3.2 ETHNOMEDICINAL WISDOM OF DIVERSE COMMUNITIES

Ethnomedicine broadly refers to the traditional medical practices concerned with the cultural interpretation of health, diseases, and illness that addresses the healthcare process and healing practices (Krippner and Staples, 2003). It is a vast interdisciplinary science that includes the knowledge about the use of natural pharmaceuticals and the ethnic group from which the same pharmacologically active ingredients belong as well. From Indian Ayurveda to Traditional Chinese Medicine (TCM) of China, from Muti of Africa to Unani medicine of Mughal India, it has been widely practiced in diverse ancient civilizations. The practitioners of

traditional medicines follow their traditions, observations, and belief but unaware about the modern theory of treatment. However, their “proof of concept” was based on the end result of using such therapy for generations. The main theme of their treatment was to provide relief to the sufferer, and then find the real cause of the suffering with the belief of “healing from within.” Those traditional practitioners did have a great knowledge about herbalism and ethnobotany as well as about human nature which may not be based on modern anatomy, physiology, biochemistry, and genetics. Species of *Hydnocarpus* were used by the ancient people of China for the treatment of Leprosy between 3000 and 2730 BC. Finding of opium poppy and castor bean from Egypt tombs revealed the use of phytomedicine in Africa as far back as 1500 BC. The *Old Testament* also mentions the use of medicinal herb and their cultivation. On the other hand, Ayurveda, the oldest surviving medical system of India about 5000 BC, uses nearly 750 plants like *Aconitum*, *Clitoria*, *Cosinium*, *Shorea*, and many more. Ayurveda is not only the rational use of medicinal plants but the central tenant of Ayur Bijnan (Science of life) is to maintain the harmony of human body and mind with all the elements of the universe, which can help in the management of life-style or microbial diseases including viral fever, meningitis, genital lesions, Amoebiasis, Leishmaniasis, high blood pressure, asthma, diabetes, and even cancer (Jiang et al., 2013). Like Ayurveda and African Traditional Medicine, ancient Chinese Traditional Medicine (CTM) also relies on harmony or balance between the body and soul in addition to uses of herbs. Traditional Chinese healers use herbs as well as other practices like acupuncture, tai-chi, and ai-gong for the treatment and/or prevention of diseases. Acupuncture involves the stimulation of specific nerve points on the body, while tai-chi and ai-gong involve gentle dance-like body movements with mental focus, breathing, and relaxation. Interestingly, Yoga, practiced by Indus-Sarasvati civilization in Northern India over 5000 years ago, mentioned in the Rig Veda, served as mainstream medical practice to maintain health and longevity. Chinese medicine, particularly CTM, also known to treat several dermatological disorders, coronary heart disease, hypertension, stroke, diabetes, atherosclerosis, etc. (Gong et al., 2017). Other schools of traditional medicines includes traditional Korean medicine, Arabic medicine, Haitian folk medicine, Uyghur traditional medicine, Celtic Medicine, Japanese Kampo medicine, and many more.

### 3.3 ETHNOMEDICINE IN INDIAN CONTEXT

India harbors a rich history of traditional medicine and a great knowledge of medicinal plants. It has been speculated that India's most known traditional medicine "Ayurveda" existed even at the time of Indus valley civilization, and reached its apex during the Vedic period due to its widespread use and significant development. Ayurveda emphasizes to maintain balance between human body, mind, and the nature. With the use of natural resources, mainly plants and minerals, Ayurveda also focuses on exercise, healthy diet, yoga, and meditation to heal any ailments or disease. One of the main pillars of Ayurveda is the medicinal resources used by ethnic communities (Ethnomedicine) and plant-based treatments using different parts of plants including roots, leaves, fruits, bark, or even seeds. The book *Yoga Ratnakara* (1700–1800 CE, unknown author) mentioned the use of opium (*Papaver somniferum*) with minerals as an herbo-mineral formula for diarrhea; while in the book *Bhaisajya Ratnavali* opium and camphor (*Cinnamomum camphora*) are used for acute gastroenteritis, and hemp/marijuana/ganja (*Cannabis indica*) for treating diarrhea. During the British rule the practice of Ayurveda was neglected by the British Indian Government. However, after the independence of India the focus was intensified on Ayurveda and other traditional medical systems. In the past two decades, Ayurveda and other traditional healthcare system such as Yoga, Naturopathy, Unani, Siddha, and Homeopathy (AYUSH) brought under a separate Ministry, and became a part of the Indian National healthcare system with establishment of dedicated colleges and hospitals. Today the AYUSH system of healthcare is running parallel with the mainstream modern medicine in India.

India is inhabited by about 645 indigenous tribes having rich knowledge of wild flora and fauna to manage or cure diseases with other miraculous use. Most of this "traditional wisdom" is undocumented and orally passed over generations. Unfortunately, each time a traditional healer dies without passing their knowledge on to the next generation, the community and the world lose irreplaceable time-tested knowledge about medicinal plants gathered over thousands of years. Indian research policy has turned its attention to validate this untapped knowledge for past few years. However, since 1994 a group of researchers first time initiate a scientific documentation along with validation study to understand why the ancient ethnic communities of Andaman and Nicobar Islands survive, mostly without the help of modern medicine. Five years of rigorous

interaction with those communities by establishing personal relationship, the researchers able to unveil some interesting insight of the healthcare culture of Onge, Nicobarese, and Shompen communities through scientific validation of a few useful medicaments which transcribed their traditional “information into modern innovation.” Chattopadhyay et al. have identified and validated a number of useful traditional medicaments of Bay Islands used for the management of infection, inflammatory condition, fever, pain, depression, contraception etc., including the antiviral activity of a useful herb *Ophiorrhiza nicobarica* collected from Galathia River basin of Great Nicobar Island, and widely used for skin ailments by the local and tribal communities (Chattopadhyay et al., 2006; Chattopadhyay and Bhattacharya, 2008). Later Bag et al. (2013, 2014) validated its potential and efficacy in cutaneously and vaginally infected animals, which lead to the identification of an alkaloid having potent anti-herpes activity at 1.1–1.5 µg/mL, much lower than acyclovir, by blocking the immediate early transcription of Herpes Simplex Virus types 1 (HSV-1) and 2 (HSV-2). While the extract of *Achyranthes aspera* and its isolated compound Oleanolic acid, used by traditional healers and some tribes of Rajasthan and Gujrat to treat asthma, boils, bronchitis, dysentery, pneumonia, skin diseases, fever, and typhoid (Goyel et al., 2007), was found to inhibit HSV-infection by blocking early stage of virus multiplication (Mukherjee et al., 2013). Similarly the stem bark of *Odina wodier*, a traditional medicine of Jangalmohal, used for curing ulcer, heart diseases and skin infection is found to prevent HSV-1-infected animals by inhibiting the viral multiplication through modulation of host immunity (Ojha et al., 2013), while the isolated chlorogenic acid regulate COX-2-dependent prostaglandin-E2 and TLR 4 signaling pathway (Ojha et al., 2014). Panda et al. (2017) have screened traditional medicinal plants used by tribes of Similipal Biosphere Reserve, Odisha and reported antiviral activity of few plants against Enterovirus-71.

### 3.4 VIRAL DISEASES—A GLOBAL HEALTH CONCERN

*An inefficient virus kills its host, while a clever virus stays with it.*

*James Lovelock*

Viruses are acellular ultra-microscopic metabolically inert obligate intracellular parasites of cellular hosts (Chattopadhyay et al., 2009b), associated with diverse diseases and are threats to human, wildlife, and livestock (Malosh et al., 2017; Marston et al., 2017; Prkno et al., 2017;

Akbari and Elmi, 2017; Vu and Misra, 2018). Many are incurable and have no effective antiviral drugs except a very few (forty) US FDA approved antiviral drugs available for the management of some viral diseases. Complicated life cycle, frequent mutability and unique disease manifestation of each virus are the main reason for the public health concern. Treatment of viral diseases is immensely challenging because of their size, genetic variation, configuration of surface molecules, invasion strategies, mode of transmission, replication and persistent nature with rapid mutability, especially RNA viruses like influenza, severe acute respiratory syndrome (SARS) coronavirus etc., with a very high mutation rate, around 1/genome/replication (Elena and Sanjuan, 2005; Elena et al., 2000). High cost and side effects of present drugs used for treatment are also major contributing factor making antiviral drug development a daunting task. The antiviral drugs Acyclovir against HSV, Zidovudine against human immunodeficiency virus (HIV), Lamivudine against hepatitis B virus (HBV), Ganciclovir against Cytomegalovirus (CMV), Amantadine against influenza, and Pegylated IFN alpha against Hepatitis C virus (HCV) have been proven to be successful at least to some extent. But majority of viruses and viral diseases including Zika, Dengue, Ebola, Marburg, Rabies, Chikungunya, Rotavirus, HIV, etc. do not have any targeted drugs or vaccines till date. The unmet need for successful antivirals can only be fulfilled by thinking *out of the box* and ancient knowledge of ethnomedicine can be a master stroke against these deadly diseases.

### 3.5 ETHNOMEDICINE AND VIRUS

Ethnomedicines have been found to be effective as antiviral therapy, due to the following perspectives:

- Plants produce thousands of compounds as secondary metabolites than they require for their survival and propagation (primary metabolites). These secondary metabolites, grouped as phenolics (flavonoids, quinones, coumarins, tannins, and anthocyanins), terpenoids (sterols, saponins, essential oils, and cucurbitacins), alkaloids, proteins, peptides, etc., are species specific and widely varied in structure and bioactivity. These compounds are foul-smelling, toxic and are synthesized mainly as weapons of defense against predators and pathogens.
- Plant extracts are being validated for antiviral activity and use in treatments owing to the fact that many viruses are intractable to the typical antivirals. Also the effective life span of most of the antiviral is limited.

- The problems of antiviral resistance, latency, recurrence, and fastidious spread of new or emerging strains, as in the case of HIV and SARS have compelled the virologists to look for better alternatives in nature, especially for people who have very little or no access to the expensive drugs (Chattopadhyay and Naik, 2007).

Plants have the miraculous treasure of numerous compounds with abilities to cure diseases and make our immunity strong. According to WHO fact sheet 2008, about 80% of population in Asia and Africa depend on plant-derived traditional medicine. Beside Asian countries, South America, Australia, and several countries of the European Union have documented ethnomedicine against various diseases including viral diseases.

The screening of antivirals from medicinal plants of traditional use is based on their wisdom, faith, availability and positive results for generations for curing ailments or diseases. Researchers are exploring those traditional plant-based medicaments to identify new source of antivirals. Most of these plants or plant-derived phytochemicals are reported to have antiinflammatory, antioxidant, antipyretic, antihelminthic, antifungal, antibacterial, and antiviral activities (Chattopadhyay et al., 2009a,b). Drug-like activities of plants or plant products, including antiviral activity, have been attributed to the secondary metabolites of plants, mainly alkaloids, flavonoids, saponins, quines, terpenes, lignans, tannins, polysaccharides, steroidal glycoside, thiosulfates, proanthocyanidin, and proteins.

### 3.5.1 Screening Models for Herbal Antiviral Agents and Their Value in Drug Discovery

- *In vitro* primary screening

There are different *in vitro* and *in vivo* methods used to study the antiviral activities of plant products, but the most commonly used *in vitro* method for preliminary screening is cytopathic effect reduction (CPE), dye exclusion and plaque reduction assay (PRA) or plaque assay. Another rapid and sensitive *in vitro* procedure of evaluating antiviral agents is based on Spectrophotometric assessment for viability of virus- and mock-infected cells. Detailed methodologies for studying antiviral activities of plant products are available elsewhere (Chattopadhyay et al., 2009a,b, 2015).

- *High throughput screening*

High-capacity antiviral screening assay in 96-well microtiter plates using tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfop henyl)-2H-tetrazolium (MTS) or 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfop henyl)-2H-tetrazolium (MTT) is



convenient as well as cost and time saving. Other methods include TaqMan PCR, and microscopic inspection of the cultures.

### 3.6 MODE OF ACTION OF PLANT-DERIVED ANTIVIRAL AGENTS

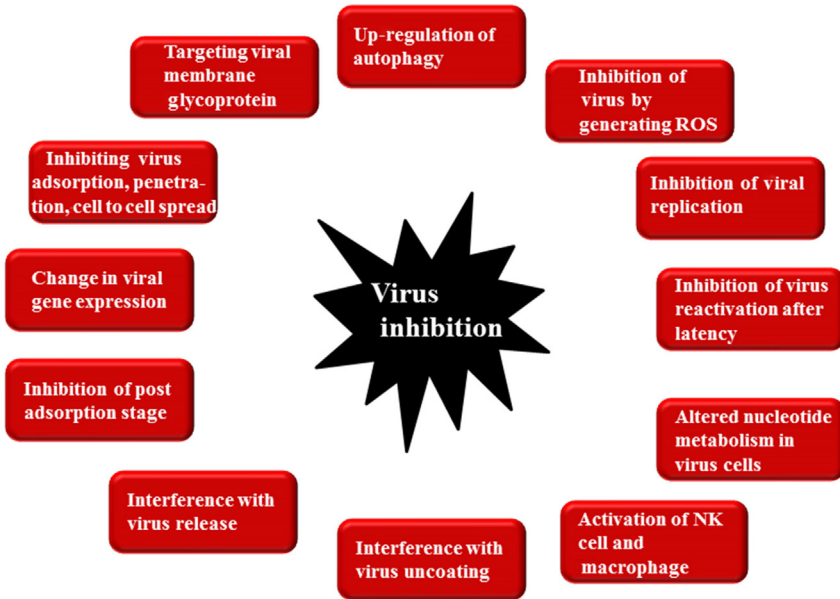
Catechin, present in the globally most popular beverage green tea leaves, when fermented into theaflavins can neutralize bovine rotavirus and coronavirus (Lin et al., 1997). Another common herb *Ocimum basilicum* or the sweet Basil of India and China has broad spectrum antiviral activity. The aqueous and ethanolic extract along with purified apigenin, linalool, and ursolic acid showed strong activity against HSV-1 (Bag et al., 2012), adenovirus 8 (ADV-8), Cocksackievirus B type-1 (CVB1) (Chattopadhyay and Naik, 2007). While Isoborneol, a monoterpene of essential oils isolated from Egyptian plant *Melaleuca alternifolia*, exhibited anti-HSV-1 activity by inactivating HSV-1 replication within 30 min of exposure (Armaka et al., 1999). Similarly *Vatica cinerea* from Vietnam is reported to inhibit HIV-1 replication (Zhang et al., 2003). The plant *Cimicifuga racemosa* (black cohosh) having antidepressant activity was also reported to have antiviral activity against human retroviruses by inhibiting HIV-1 reverse transcriptase (Sakurai et al., 2004). It is important to note that the viral diseases caused by Picornavirus and Rhinovirus do not have any drugs till date, while the plant-derived compounds chrysophenol-C, and its glycoside has virucidal effect against both group of viruses (Wei et al., 2004). Scientists have also explored Himalayan flora used in traditional medicine against viral diseases (Amber et al., 2017). Ethnomedicine from India, Pakistan, China, and Nepal has been explored as source of antivirals against Influenza virus, Rhinovirus, Adenovirus, Coronavirus, and Respiratory Syncytial virus (RSV). Different compounds including monoterpenoids, flavonoids, triterpenoids, iridoid glycosides, sesquiterpenes, benzoic acids, and phenolics have strong antiviral potential. Recent emergence of deadly dengue, which is now a public health concern worldwide, has also been shown to be prevented by plant-derived drug. Lupeol isolated from *Maytenus gonoclada* has shown activity against dengue virus (Silva et al., 2017). Extracts from *Carica papaya* have now been used for the treatment of dengue in hospitals (Ahmad et al., 2011; Dharmarathna et al., 2013) mainly to prevent the reduction on number of platelet due to platelet aggregation, although the anti-dengue or anti-aggregation of platelet activity of papaya extract has not been scientifically proved till date.

In folk medicine, papaya latex is used to cure dyspepsia, external burns and scalds, while its seeds and fruits have excellent antihelminthic and antiamebic activities. However, Chinnappan et al. (2016) have found that the leaf extract of papaya could possess a dengue-specific neutralizing effect on dengue virus-infected plasma that may exert a protective role on platelets. Luteolin, a bioflavonoid isolated from several dietary and medicinal plants, has been shown to have activity against HSV-1 (Ojha et al., 2015), dengue (Peng et al., 2017), Epstein–Barr virus (Wu et al., 2016), Japanese Encephalitis (JE) (Fan et al., 2016), and Chikungunya (Murali et al., 2015). Ginseng, a well-known medicinal herb, has been used in traditional medicine for thousands of years, and was found to be effective for treating influenza (Yoo et al., 2012) and HIV (Park et al., 2014); while in a clinical trial, Ginseng was found to help in curing HBV infection (Choi et al., 2016). Ethnopharmacological use of essential oil extract of three traditional Cretan aromatic plants in Eastern Mediterranean region and Near East claimed to be effective in the prevention and treatment of upper respiratory tract infections of bacterial and viral etiology (Duijker et al., 2015). While an Egyptian plant *Nigella sativa* extract tested against influenza patients showed better activity in people who cannot be treated with interferon-alpha (Barakat et al., 2013). A great deal of scientific research is being conducted to understand the mechanisms by which plant products exert their antiviral effects. Usually, plant-derived compounds exert antiviral effects through diverse mode and mechanism including (1) inhibition by autophagy, (2) generation of reactive oxygen species (ROS), (3) change in viral gene expression, (4) inhibition of viral entry to host cell including attachment and penetration, (5) inhibition of different steps of replication, (6) inhibition of viral release, as well as modulating the host immune parameters, which are briefly presented in Fig. 3.1.

### 3.7 MECHANISM OF ACTION OF PLANT-DERIVED ANTIVIRAL AGENTS

- *Viral inhibition by autophagy*

Pentagalloylglucose found in many traditional medicinal herbs exhibits several bioactivities including antiinflammatory, anticancer, antioxidant, and antiviral effects. It has been shown to be effective against HSV-1 by induction of autophagosomes that engulfed HSV-1 virion (Pei et al., 2011). Triterpene glycyrrhizic acid, the major



**Figure 3.1** Mode of actions of plant-derived antiviral agents.

compound isolated from *Glycyrrhiza glabra*, has also been effective against HSV by promoting autophagy (Laconi et al., 2014).

- *Inhibition of virus replication*

It has been reported that a cranberry extract known as Oximacro and its purified constituent proanthocyanidins can inhibit HSV-1 and HSV-2 replication *in vitro* and prevent the virus adsorption by targeting the viral envelop glycoprotein gD and gB (Terlizzi et al., 2016). The dichloromethane extract from *Angelica archangelica* L. fruit is a potential antiviral against HSV-1 by inhibiting viral replication (Rajtar et al., 2017). Fig. 3.1 depicts diverse mechanism of action by which the natural compounds are reported to be effective against different viruses. Resveratrol, a natural component of certain foods, such as grapes, has been shown to limit HSV-1 lesion formation by inhibiting viral replication (Docherty et al., 2005). Pentacyclic triterpenes isolated from birch bark (*Betula* species) extract of Eurasian and North America can inhibit HSV-1, especially acyclovir-sensitive and acyclovir-resistant clinical isolates of HSV (Heidary Navid et al., 2014). Sulfated polysaccharide from *Caesalpinia ferrea* can inhibit Poliovirus replication along with virus adsorption, steps after penetration and viral protein synthesis (Lopes et al., 2013). Oxyresveratrol,

purified from Thai traditional medicinal plant *Artocarpus lakoocha*, has been shown to possess therapeutic effects in mice infected with HSV-1, by inhibiting early and late phase of viral DNA replication. While the anti-HSV effects of soybean-derived isoflavonoids were reported to be due to the inhibition of viral DNA replication (Argenta et al., 2018).

- *Inhibition of virus by generation of ROS*

Oligomeric stilbenoids, polyphenolic phytoalexins from some plants including *Cannabis sativa* inhibit viral growth by generating ROS (Chen et al., 2012).

- *Change in virus gene expression*

The n-Docosanol, a behenyl alcohol (22 carbon saturated fatty alcohol) from various plants, used traditionally as an emollient, emulsifier, and thickener in cosmetics, and nutritional supplement, inhibits herpes labialis and immediate early gene expression of the HSV (Treister and Woo, 2010).

- *Inhibition of viral entry*

The sulfated galactans from the red seaweeds *Gymnogongrus griffithsiae* and *Cryptonemia crenulata* is reported to inhibit the entry of HSV-1 and HIV-2 (Talarico et al., 2004). *Mentha suaveolens* essential oil and its main component piperitenone oxide exert anti-HSV activity by inhibiting viral adsorption (Civitelli et al., 2014). Moreover, piperitenone oxide from *Mentha longifolia* could interfere with some redox-sensitive cellular pathways of host cell exploited for viral replication and thus inhibits viral growth and proliferation. While the virucidal effect of peppermint oil, the essential oil of *Mentha piperita* against HSV can be attributed to the interaction of peppermint oil with viral adsorption at the host cell surface preventing the virion to enter the host cell for establishing infection (Schuhmacher et al., 2003).

- *Interference with virus release*

Thai medicinal plant *Dunbariabella prain* interfere with HSV release (Akanitapichat et al., 2006).

### 3.8 ANTIVIRAL ETHNO-PHARMACOLOGY OF MAJOR CLASSES OF COMPOUNDS

Plants have always been a major source of various bioactive compounds. Many of them have shown anticancer, antibacterial, antiparasitic activities.

Here we will briefly discuss various groups of plant-derived compounds having antiviral activities.

- *Alkaloids*

Alkaloids are a group of naturally occurring slightly acidic compounds that mostly contain basic nitrogen atoms. More than 10,000 different alkaloids have been discovered in 300 plant families. This group of compounds has been shown to be effective against viruses like hepatitis B (Li et al., 2008), HSV (Ren et al., 2010), dengue (Hishiki et al., 2017), etc.

- *Phenolics*

Phenols, also called phenolics, are a class of compounds consisting of a hydroxyl group bonded directly to an aromatic hydrocarbon group. Phenolic compounds are classified as simple phenols or polyphenols, based on the number of phenol units in the molecule. This group is an excellent candidate for antimicrobial and antiviral research. These compounds was found to be effective against HSV (Likhitwitayawuid et al., 2005), abies virus (Chavez et al., 2006), Influenzavirus (Ha et al., 2016), and many more.

- *Coumarins*

Coumarins are organic colorless crystalline benzopyrene, naturally found in many plants. In fact its name comes from a French term for the tonka bean coumarou. Coumarins have been proven to be potent against many viruses like HIV (Lin et al., 2011b), HSV (Ghannadi et al., 2014), Dengue and Chikungunya (Gomez-Calderon et al., 2017).

- *Flavones, flavonoids, and flavonols*

Flavonoids are a class of secondary metabolites of plants and fungus, while flavones are a class of flavonoids and are synthesized in response to microbial infections. Hence, they are broad spectrum antimicrobial agents. Flavonoids represent an important natural source of antiretroviral agents, especially for AIDS therapy (Pasetto et al., 2014).

- *Terpenoids and essential oils*

Essential oils are the plant-derived phenolic compounds with a C3 side chain and at a lower level of oxidation without oxygen. The oils that are enriched in isoprene structure are called terpenes; but when they contain additional elements like oxygen they become “terpenoids.” These compounds are active against many viruses (Wen et al., 2007; Lu et al., 2015).

- *Quinones*

Quinones are a class of organic compounds, named after its prototype member 1,4-benzoquinone or cyclohexadienedione, often called as “quinone.” It has been shown to be effective against Dengue (Laurent et al., 2005).

- *Tannins*

Tannins are a group of polymeric plant phenolics. Studies show that tannins are active against many viruses including enveloped viruses like Influenza virus H3N2 and H5N3, HSV, Vesicular Stomatitis virus, Sendai virus, and Newcastle disease virus; as well as against nonenveloped viruses like Poliovirus, Coxsackievirus, Adenovirus, Rotavirus, Feline calicivirus, etc. (Ueda et al., 2013).

- *Lignans*

Lignans are polyphenols found in plants. These compounds have also shown potent antiviral action (Cui et al., 2014).

Table 3.1 represents a selected list of ethnomedicinal plants and plant-derived compounds that showed promising antiviral activities against some viruses which are of concern in recent time. Dengue, a flavivirus that causes panic in developing countries including India, has sought attention from government and global research communities to look for new treatment. The use of papaya leaf extract in dengue fever, discussed earlier, while other plant-derived compounds including pentacyclic oxindole (Reis et al., 2008), chebulagic acid (Lin et al., 2011a), punicalagin (Lin et al., 2011b), baicalcin (Zandi et al., 2012), and alpha-mangostin (Tarasuk et al., 2017) reported to have anti-dengue activity. These compounds in general can modulate host cell immune system so that the host cell can fight against the dengue virus as the virus attack immune system, invade platelet, and cause postinfection complicacy including cytokine storm, particularly in the presence of preexisting heterologous antibody due to infection by another serotype of the same virus in same individual. Hepatitis caused by HBV, a common concern for liver dysfunction worldwide, has no effective treatment or vaccine. From ethnomedicinal wisdom, compounds such as oxymatrin (Chen et al., 2016) and cinamic acid (Amano et al., 2017) showed inhibitory effect on HBV by inhibiting viral DNA replication. The herpes caused by HSV-1 and HSV-2 in 90% population, via sexual or close contact from infected to uninfected individual are of great concern due to its silent epidemic nature. In spite of available gold-standard antiviral drug acyclovir, the HSV cannot be eradicated or eliminated from the host nor can prevent latency, rather it inhibits virus

**Table 3.1** Selected Ethnomedicines and phytochemicals with antiviral activity

Plants	Extract or compound	Virus	Mechanism	Reference
<i>Peucedanum salinum</i> <i>Radix lithospermi</i>	Methanol extract Naphthaquinones Shikonin	Adenovirus (ADV) type 5 Adenovirus (ADV) type 3	Inhibit replication Inhibit apoptosis and hexon protein expression of virus	<a href="#">Gao et al. (2011)</a> <a href="#">Rajtar et al. (2012)</a>
<i>Uncaria tomentosa</i>	Pentacyclic oxindoleAlkaloid fraction	DENV-2	Immunomodulation	<a href="#">Reis et al. (2008)</a>
<i>Terminalia chebula</i> Retz	Hydrolyzable tanninChebulagic acid and Punicalagin	DENV, HCV, HCMV, HIV, RSV	Inactivate free virus particles and inhibit virus entry	<a href="#">Lin et al. (2011a)</a>
<i>Scutellaria baicalensis</i> root	Flavone Baicalein	DENV-2	By type I interferon induction and virus adsorption	<a href="#">Zandi et al. (2012)</a>
<i>Garcinia mangostana</i> Linn	Xanthones $\alpha$ -mangostin ( $\alpha$ -MG)	DENV-2	Immunomodulation	<a href="#">Tarasuk et al. (2017)</a>
<i>Ficus carica</i> Latex extracts	3,4-Dihydroxy benzoic acid, p-OH-phenyl acetic acid, N-argenine	ECV-11, HSV-1, and ADV	Replication	<a href="#">Lazreg Aref et al. (2011)</a>
<i>Halenia elliptica</i> Traditional Tibetan medicine (TTM)	2-Methyl chromones 8-methoxy-2- methyl- 4H-1-benzopyran-4- one	Hepatitis B virus (HBV)	Inhibiting HBV antigens secretion	<a href="#">Zhang et al. (2015)</a>
<i>Sophora subprostrata</i>	Matrine-type alkaloid Oxymatrine	HBV	Inhibit replication	<a href="#">Yang et al. (2012)</a>

<i>Artemisia scoparia</i>	D-Glucopyran oside Scoparamide A	HBV	Inhibit DNA replication	<a href="#">Geng et al. (2015)</a>
<i>Sophora flavescens</i> root	Matrine-cytisine alkaloid	HBV	Viral growth	<a href="#">Zhang et al. (2016)</a>
<i>Cinnamomum verum</i> bark	Cinnamic acid	HCV	Induction of oxidative stress	<a href="#">Amano et al. (2017)</a>
<i>Ficus benjamina</i> (ethanol extract of leaves)	Quercetin 3-O- rutinoside, Kaempferol 3-O- rutinoside and Kaempferol 3-O- robinobioside	HSV-1	Virus multiplication	<a href="#">Yarmolinsky et al. (2009)</a>
<i>Punica granatum</i> (many plants)	Gallic acid, gallic acid gold nanoparticles	HSV	Attachment and penetration	<a href="#">Halder et al. (2018)</a>
<i>Sophora subprostrata</i>	Propolis	HSV	Pretreated before infection	<a href="#">Nolkemper et al. (2010)</a>
<i>Mallotus peltatus</i> Leaf, bark	Pentacyclic triterpeneUrsolic acid	HSV-1, HSV-2	Inhibiting early stage of multiplication	<a href="#">Bag et al. (2012)</a>
<i>A. aspera</i>	Triterpene acidOleanolic acid	HSV-1 and HSV-2	Modulation of early immunological parameters	<a href="#">Mukherjee et al. (2013)</a>
<i>Ophiorrhiza nicobarica</i> Balkr	Indole alkaloid 7- methoxy-1-methyl- 4,9-dihydro-3H- pyrido[3,4-b] indole	HSV-2	Immediate early transcription by blocking recruitment of LSD-1 by HCF-1	<a href="#">Bag et al. (2013)</a>
<i>Caesalpinia ferrea</i>	Sulfated polysaccharide	HSV, poliovirus	Inhibit adsorption, postpenetration, and synthesis of viral proteins	<a href="#">Lopes et al. (2013)</a>

(Continued)



**Table 3.1** (Continued)

Plants	Extract or compound	Virus	Mechanism	Reference
<i>Berberis vulgaris</i> , <i>B. aristata</i> , <i>Tinospora cordifolia</i> <i>Coptis chinensis</i>	Isoquinoline alkaloids Berberine from root, stem, bark	HSV	Modulating cellular signaling, including p53 NF- $\kappa$ B, and mitogen-activated protein kinase	<a href="#">Song et al. (2014)</a>
<i>Mentha suaveolens</i>	Essential oils Piperitenone oxide	HSV	Late phase of replication	<a href="#">Civitelli et al. (2014)</a>
<i>Andrographis paniculata</i>	Diterpenoid lactone 3,19-isopropylidenean drographolide	HSV	Inhibit postentry, early gene expression; suppress ICP8 transcription, DNA replication and gD expression	<a href="#">Kongying yoes et al. (2016)</a>
<i>Curcuma longa</i> rhizome	Curcumin	HCMV	Protein expression of Hsp90 $\alpha$	<a href="#">Lv et al. (2015)</a>
<i>Angelica archangelica</i>	Xanthotoxin, Bergapten, Imperatorin, Phellopterin, Isoimperatorin (isopentenylloxy moiety at C-8 position)	HSV-1 and Coxsackievirus B3	Reduce viral titer	<a href="#">Rajtar et al. (2017)</a>
<i>Rheum</i> root	Anthraquinone derivatives Physcion, Emodin, Rhein	HIV	HIV-1 Reverse Transcriptase (RT)	<a href="#">Esposito et al. (2016)</a>

<i>Rheum officinale</i> baill	Whole extract	HIV-1	RNase H and RT-associated RNA Dependant DNA Polymerase	
<i>Schisandra chinensis</i>	Schisandrin B Deoxyschizandrin	HIV-1	Affect HIV-1 RT	Xu et al. (2015)
<i>Cassia sieberiana</i> <i>Vitex doniana</i> , <i>Croton megalobotrys</i>	Methanol extract of root and bark	HIV	Replication	Tietjen et al. (2016)
<i>Cynomorium songaricum</i> Rupr.	Polysaccharide (water soluble)	HIV	Multiplication	Tuvaanjav et al. (2016)
<i>Coffea arabica</i> seed <i>Allium sativum</i> Bulb <i>Plumbago indica</i>	Polyphenol caffeic acid Allicin and plumbagin	Influenza A virus Influenza A (H1N1)	Inhibits multiplication Viral adsorption, nucleoprotein synthesis and polymerase activity	Utsunomiya et al. (2014) Chavan et al. (2016)
<i>Laggera pterodonta</i>	Pterodontic acid	H1 subtype of Influenza A	Block nuclear export of viral RNP complexes	Guan et al. (2017)
<i>Forsythia suspense</i> (Thunb.) Vahl fruit	Forsythoside A	Influenza A	Reduction of influenza M1 protein	Law et al. (2017)
<i>Dendrobium nobile</i> <i>Rheum palmatum</i>	Dendrobine Chrysofanol and aloe-emodin	Influenza A Japanese Encephalitis	Growth IFN- $\gamma$ triggering host innate immune response	Li et al. (2017) Chang et al. (2014)
CTM hochu-ekkito (HET) or bu-zhong-Žyi-qi-tang (Chinese)	Mixture of 10 plants	MCMV	Induction of IFN- $\gamma$ and perforin-mediated cytotoxicity by natural killer (NK) cell activation	Hossain et al. (1999)
<i>Coptidis rhizome</i> Rhizome extract	Not detected	RSV	Induction of type I interferon-related signaling	Lee et al. (2017)

HCV, hepatitis C virus; RSV, respiratory syncytial virus; HSV, herpes simplex virus; DENV, dengue virus; HCMV, human cytomegalo virus; MCMV, murine cytomegalo virus; HIV, human immunodeficiency virus; HBV, hepatitis B virus; ADV, adenovirus; JE, Japanese encephalitis virus; ECV, echovirus.

replication by blocking thymidine kinase and/or DNA polymerase of the virus, and thereby promote frequent emergence of drug-resistance strains deficient in thymidine kinase which is also a great concern. HSV is used as a standard model for screening of antivirals and its easy laboratory application made it a choice for researchers to study viruses. A vast number of anti-HSV plant extracts and compounds have been cited. But majority of them are from *in vitro* studies and have been shown to prevent adsorption to cell-to-cell spread. However, latency in HSV is the real twist and we need drugs that can eradicate the virus from brain to prevent its spread. Ursolic acid (Bag et al., 2012), oleanolic acid (Mukherjee et al., 2013), berberine (Song et al., 2014), masilinic acid (Zahmanov et al., 2015), and hermaline (Bag et al., 2013) have been reported to have anti-HSV activity by inhibiting entry, immediate early, early and late replication. Similarly, antiviral plant extracts and compounds have also been reported for Influenzavirus, Poliovirus, JEvirus, and RSV.

### 3.9 CHALLENGES FOR ETHNOMEDICINES AS ANTIVIRALS

- Many of the viral diseases are incurable and do not have a vaccine yet. Discovery of safe, effective, and inexpensive antivirals from natural source, particularly from ethnomedicinal practices are among the top priorities and challenges in the future.
- Certain natural products show synergistic activities in combination with other natural products or with existing antivirals particularly useful against herpes and retroviral infections. Thus, proper combinatorial techniques to enhance the antiviral activities of these natural products need to be prioritized.
- Recombinant viral vectors mimicking infection and expressing firefly luciferase maker gene are being used in antiviral screening. Therefore, the safety issues involved while screening the antivirals are being met and should be the top most priority in future research as well (Esimone et al., 2005).
- Plants, other than being the sources of natural products, serve as efficient systems for the production of vaccines and pharmaceutical grade peptides/proteins. Several other vaccine antigens have been successfully expressed in plants, after the first subunit vaccine HBsAg was produced in 1992 (Ma Julian et al., 2003; Glenz and Warzecha, 2006).
- Isolation of active ingredient from plants is a challenge and demands continuous up-gradation of technique and laboratory infrastructure

through the years, so that those are available at an affordable cost to the public. In China and India, crude extracts are effectively used in healthcare although it is difficult to get these extracts approved by the FDA. In countries where resources are limited, government sponsored explorations will serve as a gateway for merging modern drug discovery with traditional/conventional medicine.

- Many of these natural products prevent the entry of the virus into the host cell or target specific enzymes of the virus, which may be the molecular basis of viral drug resistance. An alternative mechanism of action can be the potential target to tackle the emerging, reemerging and drug-resistant viral infections. Thus, elucidation of mode as well as mechanism of actions of the potential antivirals is equally important in research.
- Herbal medicines are being used individually or in combination, but their effects on the living systems are not scientifically documented. Results of clinical findings of using combination of plants or plant products like coadministration of kava-kava (*Piper methysticum*) and St. John's wart (*Hypericum perforatum*) lead to hepatotoxicity (Musch et al., 2006). This type of information should be available to the healthcare providers practising traditional medicine.
- It is equally important that the experiments on cytotoxicity of traditionally used plant extracts may be conducted as randomized, double-blind, placebo-controlled multicentric clinical trials so that the incorporation of a particular herbal remedy into the healthcare system is safe, besides being effective.
- Several plant species have become extinct, while many phytochemicals mentioned in the classics may have undergone change with time as well as due to anthropogenic and environmental factors. The nature of the natural compound is complex and requires a flexible standardization process from time to time.
- Screening a potential antiviral from millions of plant species is a tedious and time-taking process. A wide variety of computational techniques will help the chemists to virtually screen a huge library to a manageable size.
- Drug discovery based on ethnomedicine follow “reverse pharmacology,” where the already documented traditional plants having healing ability can be further experimented as drug candidates in clinical research. The critical pharmacopoeia tests such as the dissolution time, microbial, pesticide, and heavy metal contamination must be in accordance with the standards globally accepted protocols of antiviral screening (Chattopadhyay et al., 2009b).

### 3.10 CONCLUSION

Traditional medicines have been increasingly used by diverse communities in many parts of the world, due to its important role in maintaining good health with increasing awareness and research. According to the WHO, the goal of “health for all” cannot be achieved without the incorporation of herbal medicines in primary healthcare system. Traditional medicine has a long history in disease control and public health management. Today, an increasing number of plants used in traditional system of medicine are reported to have diverse activities in infectious diseases, particularly in viral infections, and thus can be new sources of antivirals. The age-old medicinal system is being revisited to tackle the emerging public health issues. Thus, there is a significant need to enhance drug discovery process with respect to natural products, not just for the next 10 or 20 years, but for the next  $20 \pm 40$  years and beyond, because there are new diseases waiting to discover or invade. Complex structures of phytochemicals, synergistic activity and inadequate validation could not provide the drug which can have the potential to develop as a drug or a drug candidate to inhibit or eradicate the selected viruses alone or in combination with the existing antivirals without much harm to the host. With the advent of new approaches to drug discovery, innovative strategies would be required, that will reveal and contribute the full range of chemical diversity of these valuable natural products to the drug discovery process. It is also important that the development of plants and other natural products for medicinal and other biological purposes should be potentiated on a sustainable and renewable basis in local environment. Thus, pharmaceutical companies should consider developing local resources and decentralizing selected aspects of their research operations globally. Continuous research by using the advanced technology and bowfins can gift us the lead or leads to develop novel antiviral(s) from the treasure of Ethnomedicine.

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