24 Antiviral Activity of Phytochemicals: A Current Perspective

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Key Points

- A wide variety of active phytochemicals have been found to have therapeutic applications against genetically and functionally diverse viruses.
- The antiviral mechanism of these agents may be explained on the basis of their antioxidant activities, scavenging capacities, the inhibition of DNA, RNA synthesis, or the blocking of viral reproduction, etc.
- Numerous epidemiological and experimental studies have revealed that a large number of phytochemicals have promising antiviral activities. Especially in the last decade, a number of promising leads have been identified by a combination of *in vitro* and *in vivo* studies using diverse biological assays.

Key Words: Antiviral, phytochemical, infection, replication, flavonoids, clinical trials, mechanism.

24.1 INTRODUCTION

Throughout the human history, man has been dependent on plant sources for his very basic needs (1). The use of medicinally active plants predates modern history. As per a World Health Organization estimate more than 80% of the world's population is dependant on traditional plants to meet their health requirements (2). A large number of plants used in the traditional medicine have now become a part of the modern world health care system because of their unique ability to synthesize a wide array of compounds with diverse health-related benefits (3, 4). Most recently, the introduction of plant-based products in the form of nutraceuticals and dietary supplements have made

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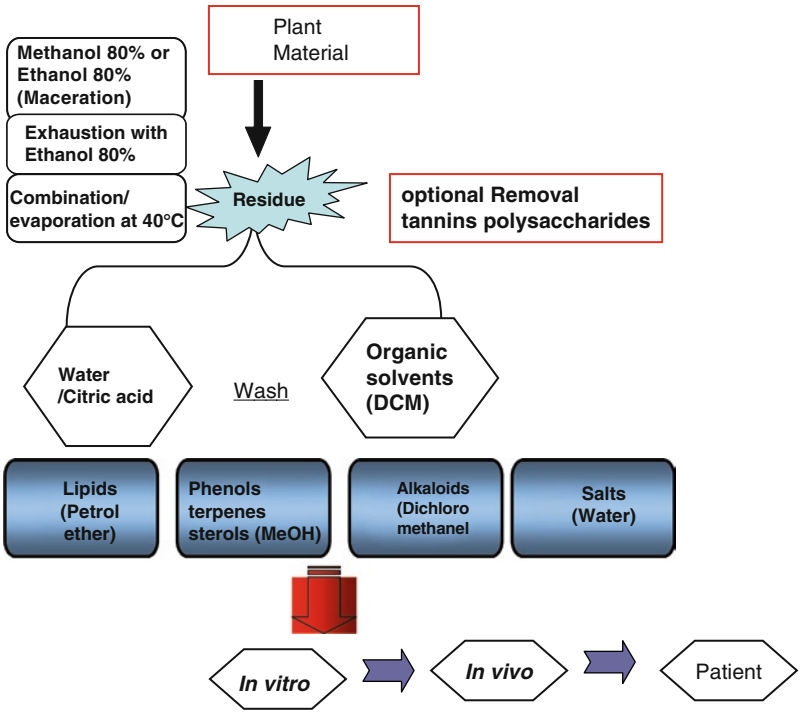

Scheme 24.1. Plants provide compounds possessing broad range of activities such as antimicrobial, antiviral, antioxidative, immunomodulatory and antitumor properties.

a major impact in the drug industry market (5, 6). The isolation, structural elucidation, and evaluation of all major constituents of the plant-based products must occur efficiently in order to determine its pharmacological properties. A general isolation and evaluation procedure of plants-based active ingredients is depicted in Scheme 24.1. Plants provide compounds possessing a broad range of activities such as antimicrobial, antiviral, antioxidative, immunomodulatory and antitumor properties (7, 8). Because of the rapid advancement in modern day biology, drug discovery from natural sources has evolved into a highly multidisciplinary field utilizing various sophisticated methods of isolation, analysis, and evaluation. Over the last few decades, natural products have been studied for anti-infective and more specifically, antiviral activities. Basic researches in experimental models using various biological systems strongly suggest the protective role of plant-derived natural compounds against different viral infections (9, 10). Despite the substantial amount of progress made in the treatment and therapeutic strategies, the incidence, morbidity, and mortality of viral infections remain a major global challenge. The conditions are more complicated in the developing world due to the unavailability of relatively expensive medicines and widespread drug resistance (11). Unfortunately, many antiviral compounds presently in clinical use have a relatively narrow spectrum of activity, limited therapeutic usefulness, and variable toxicity. Whether natural antivirals can be developed as a viable alternative medicine or a synergistic combination therapy with pre-existing antiviral therapy will entirely depend on identifying broad-spectrum plant-based antivirals combined with a method of delivery and keeping in mind its stability and bioavailability. In addition, the development of a

suitable *in vitro* pharmacodynamic screening technique could contribute to the rapid identification of potential bioactive plants and also to the standardization and/or pharmacokinetic–pharmacodynamic profiling of the bioactive components.

Originating from nucleosides or closely related carbon structure, the wellknown drugs face an emerging problem of development of resistant viral strains (12, 13). In addition, the emergence of many new and perhaps more deadly viruses such as *Ebola and Marburg* viruses and the possible threat of their use as arsenal for bioterrorism have enhanced our urgency to find new and potent antivirals as soon as possible. This need is further aggravated by the fact that viral infections are now recognized as the second most important known cause of human cancer (14). Viruses absolutely require host cell environment for survival. Besides the genetic variation, divergent invasion strategies pose a major challenge. Since medicinal plants have an endless variety of chemical constituents, it could be utilized to counter genetic and invasion divergence and thus inhibit the replication of both DNA and RNA virus. In fact, the world of ethnopharmacological knowledge increases the success probability of finding a new drug candidate (15, 16). In this article, we examine current developments on various naturally occurring antiviral compounds. The major advances in the field of virus growth inhibition have been summarized. In addition, the origin, mechanistic action, and phase trials of various plant-derived antiviral agents have been included in this chapter.

24.1.1 Antiviral Assays

In the process of drug discovery, the selection of an appropriate bioassay and its validation are the important steps to determine the activity of the products and extracts. The determination of antiviral efficacy is relatively complex because it is not possible to design a single assay for different viruses as they require different cell systems. The fact that so many so-called "exciting molecules" do not graduate to the next stage is primarily due to major flaws in the screening methodologies. Some of the major challenges include using high assays dosages, using improper test controls, and the wrong selection of targets and endpoints. In spite of the progress made, little attention has been given to the influence of various reaction parameters (17). The lack of standardization in methodologies produces highly inconsistent results posing a major obstacle in developing a novel entity as a drug molecule. More commonly, the evaluation of the antiviral is based on the capability to replicate in a particular cell system.

The efficiency of the plant extract can be evaluated by large number of methods. At a preliminary level, the *in vitro* efficacy is detected using markers such as cytopathic effect, plaque formation, or proliferative effects on diverse cell lines (Table 24.1). The detection of viral RNA and DNA do provide information about the viral replication. Although a number of assays have been developed there is still a need for more standardized assays to provide consistent results. The complexity in evaluation of viral inhibition is attributed to its efficient replication coupled with its genetic variation and diverse invasion strategy. Confluent monolayers of the cells are infected with virus in combination with a varied concentration of the plant extract and incubated followed by the calorimetric determination of viable cells. Radioactive-labeled viruses are employed to determine the mode of antiviral activity (18). Determination of the values of EC_{50} (reciprocal dilution required to prevent virus-induced cytolysis by 50%) and $TCID_{50}$ (reduction of viral titer) are used as a measure to determine viral activity.

Table 24.1
Invitro efficacy assay

	Details about the virus	*Assay type*	*Assay methods*
1	Plaque formation capability	Plaque inhibition Plaque reduction	Titer determination in the presence of non-toxic dose of compound
2	Cytopathic effect inducement capability	Inhibition of the viral-induced CPE Virus yield reduction Endpoint titer determination	CPE determination with limited dose of virus Virus yield under treatment with a given amount of virus Virus titer reduction determination after dilution
3	Negative plaques formation cytopathic effect capability	Specific function determination	Hemagglutination test Hemadsorption test Inhibition of cell transformation Immunological tests: detection of antiviral antigens in cell culture (HSV, CMV, EBV, HIV)
4	Miscellaneous tests	Nucleic acid/ polypeptide inhibition Radioisotope uptake study Genome number determination	Reduction or inhibition of viral specific nucleic acid /polypeptides synthesis in infected culture Determination of uptake of radioisotope labeled precursor Viral genome copy with single compounds or with mixtures

24.2 CLASSIFICATION OF ANTIVIRAL PHYTOCHEMICALS

24.2.1 Flavonoids

The flavonoid structure, basically a polyphenol consisting of 15-carbon atoms skeleton (C_6–C_3–C_6 system) (**1**), constitutes the largest source of antiviral agents in the entire plant kingdom. In some compounds, the C_2 carbon atom is directly linked to the oxygen as a result of which furan type molecule is formed called aurone (**2**). The further subclassification of flavonoids is based upon the oxidation and the substitution pattern of the ring C. The biochemical effects of flavonoids are attributed to their ability to inhibit the number of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca^{+2}-ATPase, lipoxygenase, cycloxygenase, etc., besides the regulatory role on different hormones like estrogens, androgens, and thyroid hormone (19, 20). Evaluating flavonoids for activity against herpes simplex virus (HSV), Thomas et al. reported that flavonols are more active than flavones (galangin > kaempferol > quercetin) (21). Flavonoid-based polymer (MW 2100 Daltons) has displayed substantial activity against HSV type-1 and type-2 strains (22). On the basis of the evaluation of a flavonoid subset, Gerdin et al. found that flavan-3-o1 was more effective in selective inhibition of human immunodeficiency virus (HIV)-1, -2, and similar immunodeficiency virus infections (23).

Chalcones, having general formula ArCH=CHC(=O)Ar forms the central core for a variety of important biological compounds. Considered as precursors of flavonoids and isoflavonoids, these compounds are abundant in edible plants, and display a diverse array of pharmacological activities. Deng et al. have reported excellent antiviral activity of chalcones **3** and **4** utilizing pharmacophore models to identify chemical signatures considered important for the antiviral activity (24, 25). Dihydrochalcones (**5**) (obtained by double bond reduction of chalcone) derived from *Millettia leucantha KURZ* (*Leguminosae*) showed anti-herpes simplex virus (HSV) activity (26). Flavones, structurally characterized by 2-phenylchromen-4-one backbone, are found in *Lamiaceae*, *Apiaceous*, and *Astraea* families. Likhitwitayawuid et al. described the isolation and anti-HSV activities of a series of phenolic compounds identified from the heartwood of *Artocarpus gomezianus*, including the new antiherpetic flavone artogomezianone (**6**) (27). Prendergast et al. have used 3′,4′-diacetoxy-5,6,7-trimethoxyflavone or naringin (**7**) in the treatment of viral (e.g., HCV, HIV, a picornavirus genus virus or a respiratory virus) or parasite (e.g., toxoplasmosis) infections (28). On the basis of molecular electrostatic potential (MEP) maps, Mishra et al. proposed that the anti-picornavirus activities of the flavones are related with negative MEP values in two regions, one near the 3-methoxy group and another in a diagonally opposite region near the substituent attached to the C_7 atom of the molecules (29). We have synthesized and confirmed the antiviral activity of several novel analogs of flavanone Abyssinone II (**8**), a naturally occurring prenylated flavanone, in HeLa cells using a recombinant β-galactosidase expressing strain of HSV-1(Herpes simplex virus Type 1) (30). Characterized by hydroxyl group at position C_3 of the flavonone molecule (**9**), flavanol mixture is applied for treating and preventing hepatitis B, mycotic infection, liver protection, inflammation disease, and autoimmune disease (31). The effect of several naturally occurring dietary flavonoids including quercetin (**10**) on the infectivity and replication of herpes simplex virus type 1 (HSV-I), polio-virus type 1, Para influenza virus type 3 (Pf-3), and respiratory syncytial virus (RSV) were studied in cell culture monolayers employing the technique of viral plaque reduction. Quercetin caused a concentration-dependent reduction in the infectivity of each virus. In addition, it reduced intracellular replication of each virus when monolayers were infected and subsequently cultured in a medium containing quercetin (10). Myricetin (**11**), a bioflavonoid whose occurrence in nature is widespread among plants showed excellent antiviral effect against hepatitis B virus, influenza virus, and/or coronavirus (32). Anthocyanidin (**12**) is an important group of plant pigments having free OH group which can co-ordinate with metal ions like Ca^{2+} and Mg^{2+} under alkali conditions. This coordination ability is one the major reasons for the bioactivity of molecule. Anderson et al. have reported the therapeutic effect of anthocyanidin in the treatment of diseases caused by viruses (33). Isoflavonoids is an important class of flavonoids with impressive biological activities formed as a result of migration of phenyl group from 2 to 3 as shown in **13** (Fig. 24.1). In contrast to most other flavonoids, isoflavones (**14**) have a rather limited taxonomic distribution and occur mainly within the *Leguminosae* family. Antiviral activity on Newcastle disease virus was examined and rotenone (**15**) showed significant inhibitory effects on the viral growth in cultured cells as determined by the plate and tube assay methods (34). Isoflavanones bear the same relationship to isoflavones as flavanones do to flavones. And, as in the case of flavanones, isoflavanones have a chiral center (C_3 in isoflavanones). PMZ-1, a prenylated isofla-

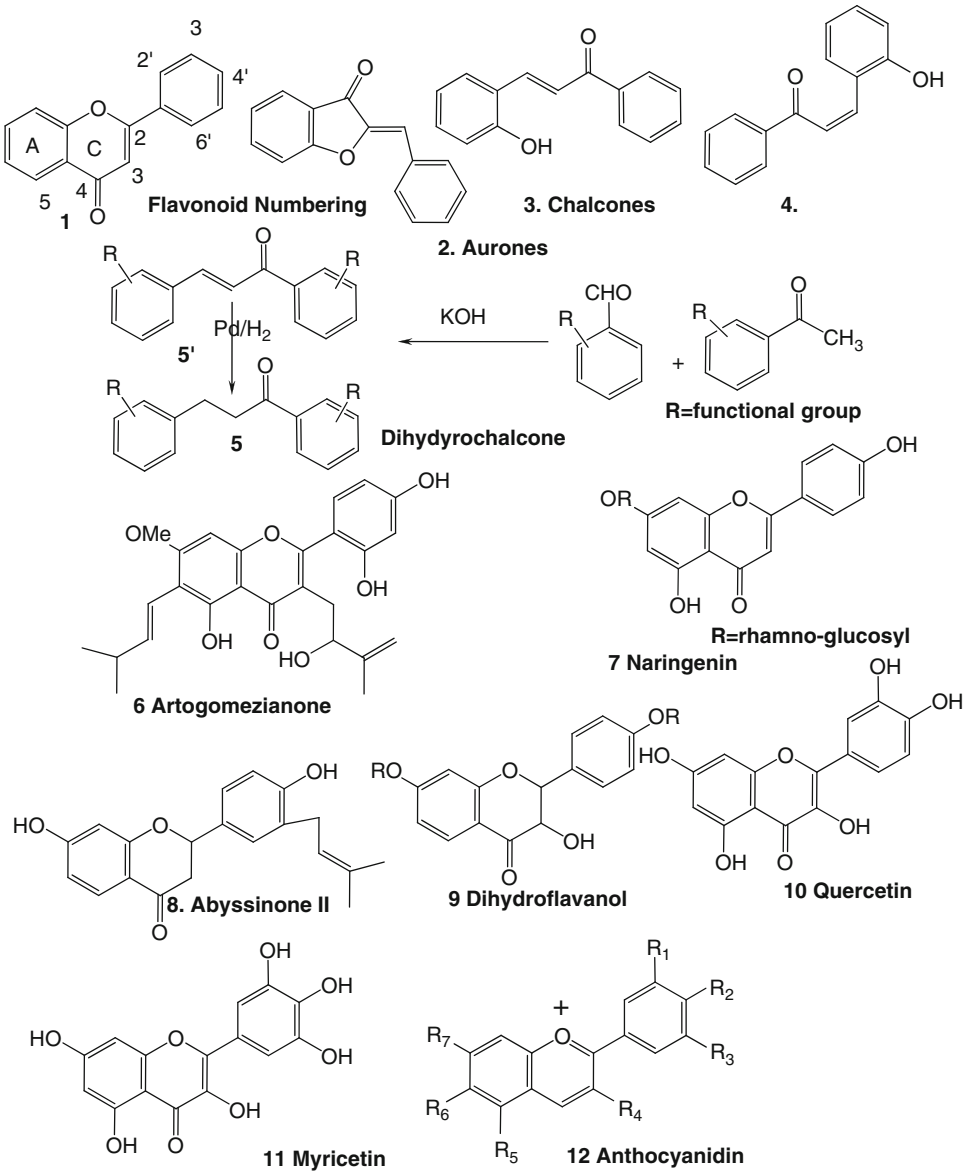

Fig. 24.1.

vonone (**16**), isolated *Bolusanthus speciosus* (Bolus Harms) has exhibited excellent activity against HIV–having a broad therapeutic index (TI > 300) (35).

Structurally, one of the simplest members of these subclasses, the isoflavans, is characterized by the fact that they do not have the carbonyl group at C_4 carbon, for example, 7,4′-dihydroxyisoflavan (**17**, equol). The effect of substituted isoflavans (**18**) (R and R1 = H, Cl, or Br) and isoflavenes (**19**) on human rhinovirus (HRV) 1B infection of HeLa cells was examined by Conti and coworkers who found that these compounds inhibited virus plaque formation in cell cultures with isoflavans being more effective than isoflavenes (36). It was found that the cells pretreated with compounds before challenge

with HRV-1B exhibited resistance to the virus-induced cytopathic effect. Arylcoumarins related to flavonoids biogenetically are characterized by the presence of a carbonyl function at C_2 and may or may not have oxygenation at C_4. A large number of coumarins has been studied for antiviral activities (37). Calanolide A (**20**) first isolated from a tropical tree (*Calophyllum lanigerum*) in Malaysia is one of the novel non-nucleoside reverse transcriptase inhibitor (NNRTI) with potent activity against HIV-1 (38).

Compounds belonging to the flavans class are normally devoid of carbonyl group at position 2. Although this class of compounds contains some common and comparatively simple compounds, catechin and epicatechin, in particular, the overall structural complexity of the group is impressive. Two new antiviral flavan derivatives were isolated from a methanol extract of leaves of *Pithecellobium clypearia* as guided by antiviral assays (7-*O*-galloyltricetifavan (**21a**) and 7,4-di-*O*-galloyltricetifavan (**21b**) (39). Neoflavonoids constitute a group of flavonoid derivatives that have their aryl group attached to C_4 as opposed in flavonoids and C_3 in isoflavonoids. A series of inophyllums **22**–**25** were isolated from the Malaysian tree *Calophyllum inophyllum* and evaluated for inhibitory activity against HIV-1 reverse transcriptase (RT). Among them, the most active compounds, inophyllum B and inophyllum P showed IC50 values against RT of 0.038 and 0.130 mM, respectively (40) (Fig. 24.2).

24.2.2 Alkaloids

Synthesized by plants from amino acids, alkaloids contain nitrogen in a heterocyclic ring. Some of the major nuclei found in various alkaloids have been shown in **26**, **27,** and **28**. Thirty-six alkaloids isolated either from *Catharanthus roseus* or *C. lanceus* were evaluated for *in vitro* activity against vaccinia and polio type III viruses. Nine of these alkaloids were effective as antiviral agents, with pericalline (**29**) being the most effective (41). In an attempt to obtain SAR data, Houghton et al. tested several naturally occurring chromone alkaloids (derived from the rootbark of *Schumanniophyton magnificum*) for the inhibition of HIV and HSV infections in C8166 and Vero cells, respectively. The authors also synthesized acyl and methyl derivatives for screening. It was found that the presence of a piperidine ring and free hydroxyl groups on the molecules seems to favor the anti-HIV activity. Irreversible binding to gp 120 was considered to be responsible for the anti-HIV activity (42).

24.2.3 Terpenoids

The terpenoids, sometimes referred to as isoprenoids, are a large and diverse class of naturally occurring phytochemicals derived from five-carbon isoprene units, which are assembled and modified in thousands of ways. Numerous phytocompounds that were evaluated for activity against anti-severe acute respiratory syndrome-associated coronavirus (SARS-CoV) activities using a cell-based assay measuring the SARS-CoV-induced cytopathogenic effect on Vero E6 cells and compounds (**30–32**) showed excellent activities (43). More than 220 phytocompounds (including ten diterpenoids, two sesquiterpenoids, and two triterpenoids) were screened for activity against anti-SARS-CoV activities utilizing a cell-based assay measuring SARS-CoV-induced cytopathogenic effect on Vero E6 cells. The bioactive compounds with anti-SARS-CoV activity in the μM range included abietane-type and labdane-type diterpenes sesquiterpenes and lupane-type triterpenes.

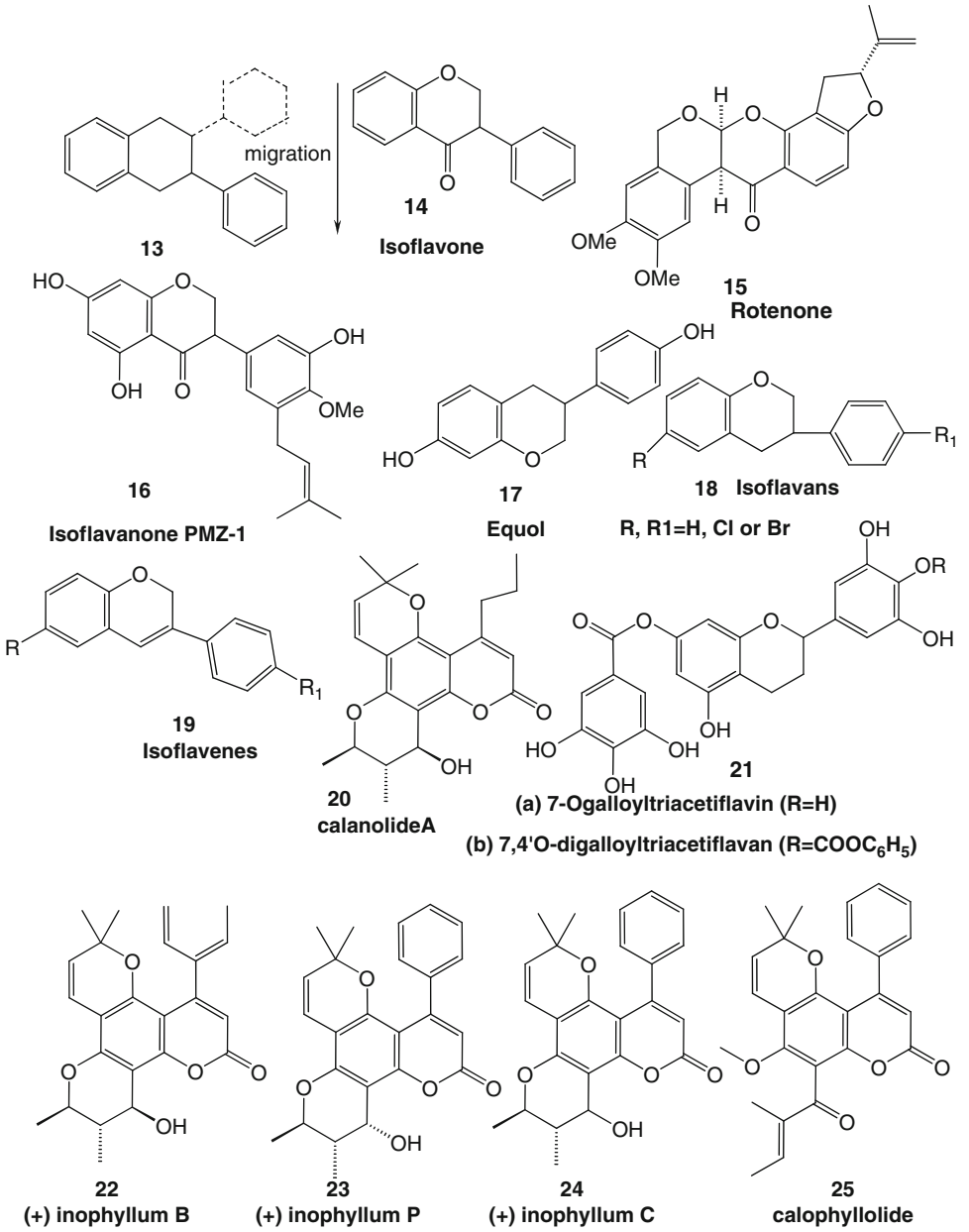

Fig. 24.2.

24.2.4 Carotenoids

Carotenoids considered as the structural backbone of compound belong to the category of tetraterpenoids (hydrocarbons resulting from the association of several isoprene units). Majority of the carotenoids are derived from the 40 carbon polyene chain which is sometimes terminated by rings. Carotenoids can be Xanthophylls (molecules containing oxygen) such as lutein and zeaxanthin and carotenes (the unoxygenated or oxygen free carotenoids). The concentrations of plasma carotenoids (α-carotene (**33**), β-carotene

(**34**), lutein/zeaxanthin (**35**) and lycopene (**36**) have been associated with the increased risk of death during HIV infection among infants in Uganda (44).

24.2.5 Organosulfur Compounds

Sulfur-containing compounds are present in all *Brasicaceae* family vegetables. In addition, plants belonging to *the Allium* family constitute an important class of antiviral agents (45). There a are number of representative examples of organosulfurs antivirals (**37–40**) that is, cauliflower, cabbage, kale, bok choy, brussels sprouts, radish mustard, and water garden cress that constitute the rich source of organosulfur compounds. Several unsymmetrical aralkyl disulfides, were synthesized and oxidized to study the relatively unexplored class of thiolsulfinate (46). The pungent odor, and chemical instability of these compounds make animal studies difficult; hence, structural modifications have been carried out. We have synthesized and screened several sulformates (based upon brassinin and sulfuraphane structures) derivatives for their HSV activities (47) (Fig. 24.3).

24.2.6 Vitamins

It has been shown that vitamin C (**41**) can increase the host immune response, and this may provide protection against infectious diseases (48). Vitamin E supplementation might be effective in the treatment of chronic hepatitis B (49). The name vitamin E covers a collection of eight fat soluble compounds, tocopherols (**42**) (methyl derivatives of tocopherol) and tocotrienols (**43**).

24.2.7 Selenium Compounds

A significant number of studies has indicated the importance of selenium compounds (**44–46**) as potent antiviral agents. The data generated from experimentation on various animal models and *in vitro* models demonstrate significant beneficial effects of selenium on different viral infections. Cermelli et al. studied the antiviral effects of three selenium compounds on the replication of Coxsackie virus B_5 replication (50). Selenite was shown to reduce viral replication in Coxsackie virus B_5 replication, but selenate and selenomethionine did not exhibit any substantial antiviral activity. Waotowicz et al. synthesized and tested different analogs of ebselen for their activity in *in vitro* antiviral assay. Some of the analogs tested had an appreciable inhibition of cytopathic activity of HSV-1 and encephalomyocarditis virus—EMCV (10) (Fig. 24.4).

24.2.8 Miscellaneous

Curcumin (**47**) derived from turmeric and a key constituent of food in the Indian subcontinent has shown potent activity against HIV-1 integrase (51). Chlorophyllin (CHLN) (**48**, **49**), a synthetic derivative of chlorophyll has been assayed for its capacity to prevent nuclear fragmentation (NF) in HEp-2 cells infected with poliovirus (52). Carboxymethyl chitin, a polysaccharides polymer containing partially deacetylated aminosugar showed a significant inhibition of Friend murine leukemia helper virus (F-MuLV) and HSV (53). Seven ellagitannins isolated from *Phyllanthus myrtifolius* and *P. urinaria*

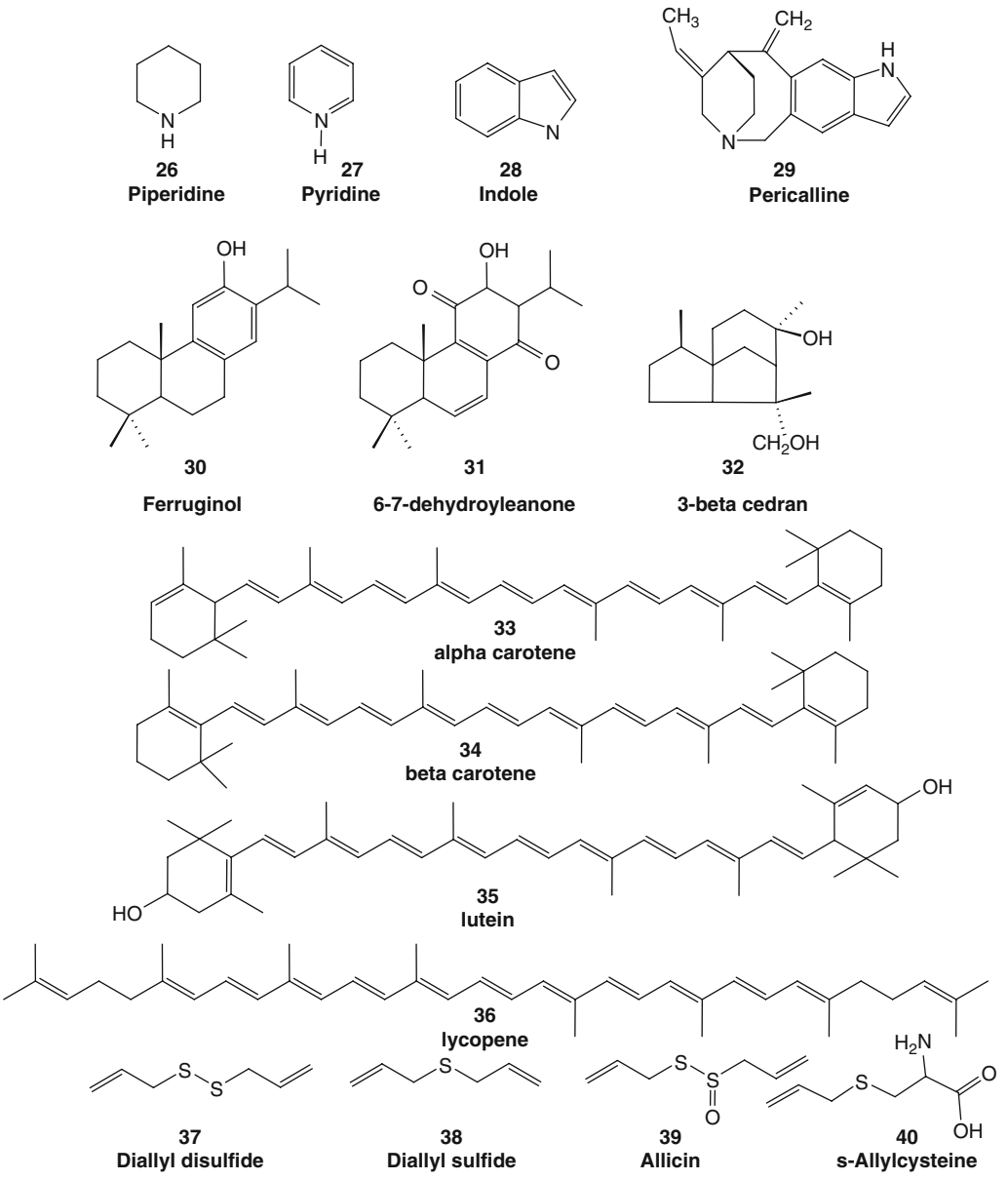

Fig. 24.3.

(*Euphorbiaceae*) have shown to be active against Epstein–Barr virus DNA polymerase (EBV-DP) (54). Polyacetylenes (**51**, **52**) are hydrocarbons that strongly absorb long-wave UV light. The medicinal activity of these compounds is altered upon exposure to light (photoactivation). The principal constituent in the leaf of *Bidens pilosa*, phenylheptatriyne (PHT), is one of the polyacetylenes that has been widely studied for its antiviral effects that is augmented by UV light exposure (55). The polyacetylenes are one of the few natural substances reported to inhibit CMV, a type of herpes virus that causes disease

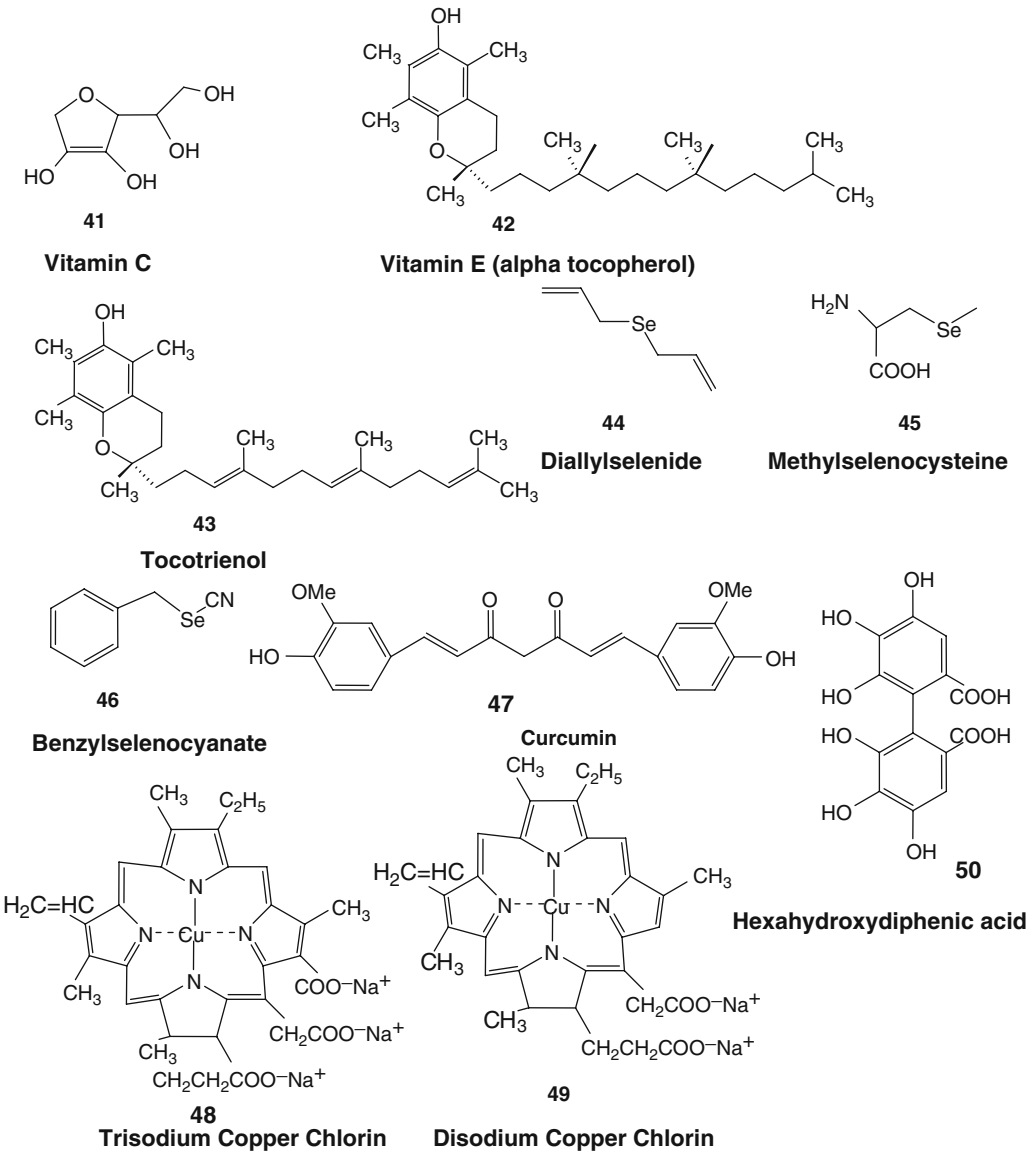

Fig. 24.4.

in immune-compromised individuals. Importantly, these polyacetylenes do not cause DNA changes (as do other herbal photoactivated substances, such as furanocoumarins found in the *Umbelliferae* plants), and the action appears to be mediated by cell surface activities, this implies a higher level of safety for their use (56).

Highly sulfated red algal polysaccharides ($C_n(H_2O)_n$ (**53**)) extracted from *Gelidium cartilagineum* afforded protection against animal virus, influenza B, and mumps viruses (57). The cell-wall sulfated polysaccharide of the red microalga *Porphyridium* sp. has impressive antiviral activity against *Herpes simplex* viruses types 1 and 2 (HSV1,2) and

varicella–zoster virus (VZV) (58, 59). Lignans are one of the major classes of phytoestrogens which are estrogen-like chemicals and also act as antioxidants (60). Nordihydroguaiaretic acid (NDGA) (**54**), a lignan present in the perspired resin of leaves of *Larrea divaricata* has displayed significant *in vitro* inhibition against several viruses, including HIV, HSV-1 and -2, and human papilloma (61). The pharmacokinetics and metabolism of retrojusticidin B, an anti-HIV reverse transcriptase agent isolated from *Phyllanthus myrtifolius*, have been studied in rats. Chrysophanic acid (**56**) (1,8-dihydroxy-3-methyl anthraquinone (**55**)), isolated from the Australian aboriginal medicinal plant *Dianella longifolia*, has been found to inhibit the replication of poliovirus types 2 and 3 (*in vitro* SARS-CoV spike (S) protein, a type I membrane-bound protein, is essential for the viral attachment to the host cell receptor angiotensin-converting enzyme 2 (ACE2) (62). Emodin (**57**), derived from genus *Rheum* and *Polygonum*, was shown to significantly block the S protein and ACE2 interaction in a dose-dependent manner. It also inhibited the infectivity of S protein-pseudotyped retrovirus to Vero E6 cells. These findings suggested that emodin may be considered as a potential lead therapeutic agent in the treatment of SARS (63). Gingerols (**58**) (derived from ginger, a typical south Asian spice) has traditionally been used to cure common colds and throat infections and form an important constituent of Ayurvedic formulations. There have been numerous studies on the efficacy of these compounds as antiviral agents (64). Salicylic acid ((**59**) $C_6H_4(OH)CO_2H$) can stimulate the inhibition of all three main stages in virus infection: replication, cell-to-cell movement, and long-distance movement. There is evidence that SA may stimulate a downstream pathway, leading to the induction of mechanism of resistance based on RNA interference (65) (Fig. 24.5).

24.2.9 Activity of Extracts/Mixtures Preparation

Traditional medicine system (Egyptian, Ayurvedic, Chinese, Unani) have utilized the plant extracts/mixtures to cure infections. The underlying idea is to achieve the synergistic or combination benefits of the formulation. In addition, herbals offer a less toxic alternative to conventional therapies thereby encouraging patients to opt for this treatment. Semple and colleagues reported that Chrysophanic acid (1,8-dihydroxy-3-methyl-anthraquinone) (isolated from the Australian Aboriginal medicinal plant Dianella longifolia) inhibits the replication of poliovirus types 2 and 3 (Picornaviridae) *in vitro* (66). Terpenes and phenol esters from *Plectranthus strigosus* were screened against herpes viruses. The bioactivity study revealed herpetic inhibitory properties for ent-16-Kauren-19-ol ent-16-kauren-19-oic acid. The compound inhibited poliovirus-induced cytopathic effects in BGM (Buffalo green monkey) kidney cells at a 50% effective concentration of 0.21 and 0.02 g/mL for poliovirus types 2 and 3, respectively. *Phellodendron amurense* bark extracts were examined and substantial antiviral activity was reported against HSV-1 utilizing the plaque inhibition assay (67). Propolis, a crude extract of the balsam which contains terpenoids, flavonoids, benzoic acids, esters, phenolics, has been found to inhibit the hemagglutination activity of inflenza virus, acyclovir resistant HSV-1, adenovirus-2 VSV, and poliovirus (68). In a study, the *Dryopteris crassirhizoma* extract was used to inhibit the reverse transcriptase associated DNA polymerase and RNAse H activity (69). An extract derived from *Asimina triloba*, has been used for the treatment of oral herpes (HSV-1) (70). Sixty-five

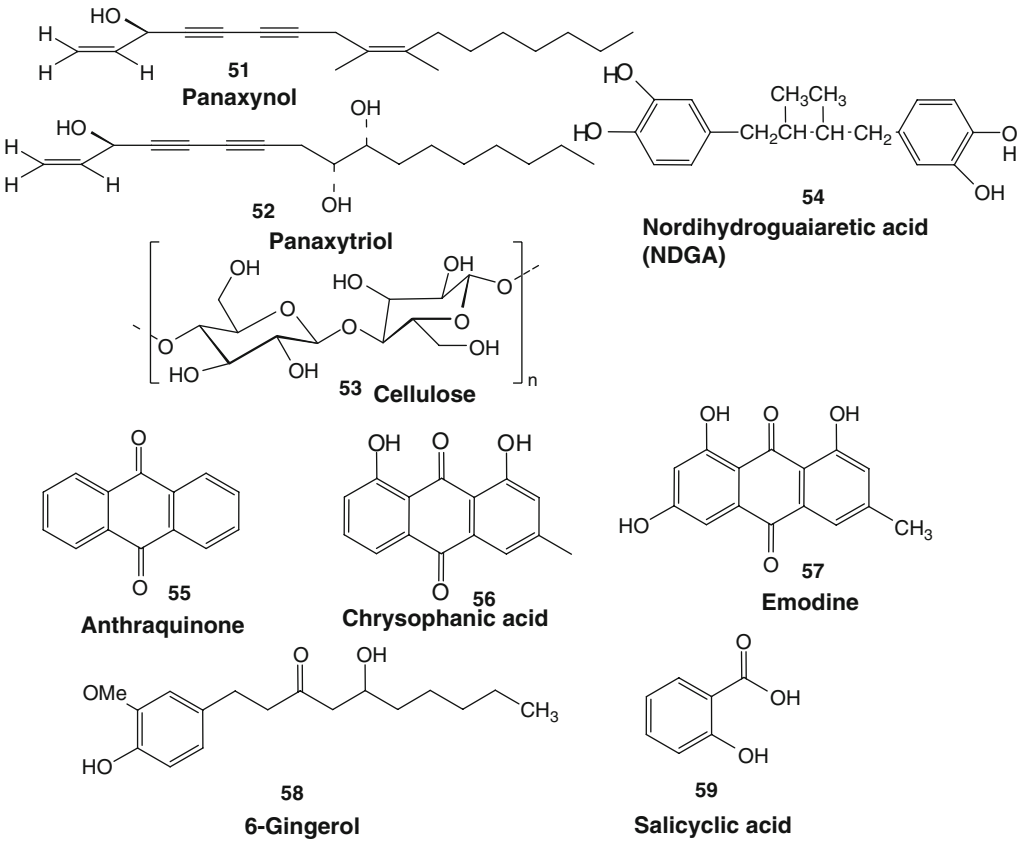

Fig. 24.5.

crude extracts from 51 selected endophytic fungi isolated from *Garcinia* species were tested for various bioactivities. Eighty percent of the fungal extracts from fermentation broths and mycelia displayed antiviral activity (71). In a related study, organosulfur compounds derived from garlic extract protected CD4 cells from HIV attack (72). Tertagalloyl glucopyranose obtained from *Juglans mandshurica* inhibited reverse transcriptase and RNAse H activity while extracts of *Centella asiataca* and magniferin of *Magnifera indica* have shown promising anti-herpes HSV activity (73). The crude extract of the roots from the Australian medicinal plant *Dianella callicarpa* (Liliaceae) displayed significant antimicrobial and antiviral activities (74). Meliacine (a partially purified extract (meliacine) from the leaves of *Melia azedarach L*) exhibits a potent antiviral effect against several viruses without displaying cytotoxicity (75). The *in vitro* antiviral activity of the Cuban-endemic plant *Phyllanthus orbicularis* against HSV-1 and -2 was confirmed and it was found that the drug acted at early stages of herpesvirus replication cycle (76). The concurrent use of natural health products (NHPs) with antiretroviral drugs (ARVs) is widespread among HIV-infected patients; however, extreme caution should be exercised since some NHPs are complex mixtures and are likely to contain organic compounds that may induce and/or inhibit drug metabolizing enzymes and drug transporters.

It has been observed that *St. John's wort* clearly induces cytochrome P450 3A4 and P-glycoprotein. This reduces protease inhibitor and non nucleoside reverse-transcriptase inhibitor concentrations, thereby increasing the likelihood of therapeutic failure (77).

24.2.10 Antiviral Mechanistic Aspects of Phytochemicals

One of the major steps in drug discovery is to identify and validate specific molecular targets. The advance of modern day biology has enabled us to identify microbial enzymes, receptors and molecular processes that facilitate drug action against a particular kind of virus. Studies have indicated that the antiviral action of plant-derived products may be attributed to a number of well-defined mechanisms (Table 24.2). It is possible that the antiviral effect of the compound may be explained on the basis of more than one mechanism and in some cases the action of mechanism may be unknown. Understanding the mechanistic pathways may help us to progress rapidly with more rational drug design and screening procedures.

24.2.11 Viral Studies

There have been numerous *in vitro* studies supporting the antiviral activity of phytochemicals. In order to further evaluate the modulation of several of these plant-derived compounds by components of tissue and body fluids, several *in vivo* studies have been carried out. However, the relative proportion of these studies is less for obvious reasons. There is tremendous amount of literature available regarding antiviral potential of phytochemicals. For the sake of clarity, the discussion has been classified into different sections with a focus on viral diseases.

24.2.12 AIDS

HIV is a retrovirus that can lead to acquired immunodeficiency syndrome (AIDS), a condition that is characterized by the failure of the immune system. According to a report by the World Health Organization, it has been estimated that 0.6% of the world's population is infected with AIDS. Until the year 2006, AIDS has killed more than 25 million people, since it was first recognized in 1981 (107). With the recent advances in understanding the biology of HIV, there has been increased focus on the usage of phytochemicals as antivirals against HIV. Owing to the vast array of chemical entities in nature, effective therapies for HIV infection are being sought in the natural world. The scope of studies of anti-HIV plant extracts is too extensive. Owing to the size limitations of the present review, we have summarized some of the major studies in Table 24.3.

24.2.13 Poliomyelitis

Poliomyelitis, caused by a human enterovirus, damages the nervous system and causes paralysis. The disease is normally prevalent in less developed Asian and African countries where polio immunization for children is not very common in spite of the massive immunization by the governments and non-governmental organizations. A large number of plant-derived products have been evaluated for their activity against

Table 24.2
Phytochemicals and antiviral activity

	Name of the phytochemicals/class	*Details of Study*	*Virus*	*Mechanism*	*References*
1	Flavone (4′, 5-dihydroxy 3,3′,7-trimethoxy flavone)	Effect on replication	Human: Picornaviruses Rhinoviruses Coxsackieviruses	Replication inhibition, selective inhibition of viral RNA	Ishitsuka et al. (78)
2	Polyphenolic complex (PC) containing: Catechins Flavonoids Kaempferol Myricetin Monne Quercetin Ramnasin Retusin	Effect of PC on the expression of viral proteins haemagglutin (HA), neurominidase (NA) and nucleoprotein (NP)		Inhibition of protein synthesis and synthesis of viral proteins	Serkedjieva (79)
3	Quercetin Quercetin 3-methyl ether Quercetin 7-methyl ether Quercetin 3,7,3′4′-tetramethyl ether Galangin 3-methyl ether Morin Robinin Quercetin 3,7,4′-trimethyl ether Quercetin 7,4′-dimethyl ether 7,4′-di-*O*-benzolquercetin 7-hydroxy-3,4′-dimethyl flavone 6,3′-dihydroxy-4′-methyl aurone Fisetin 4′-methyl ether	Effect on tomato ringspot nepovirus (TomRSV), infectivity in *Chenopodium quinoa*	Tomato Ringspot Nepovirus (TomRSV)	Proposed interference with an early event in the virus life cycle	Malhotra et al. (80)

(continued)

Table 24.2
(continued)

	Name of the phytochemicals/class	*Details of Study*	*Virus*	*Mechanism*	*References*
4	BCA, BA	Elucidation of mechanism of the antiviral effect of BA	HIV-1	Inhibition of HIV-1 infection (viral entry). Similar inhibition by Baicalein (BCA)	Li et al. (81)
5	BCA, Genistein	Investigation of antiviral activity of baicalin and genistein against human cytomegalovirus	HCMV	Blockage of HCMV infection at entry while the primary mechanism of action for genistein may be to block HCMV immediate-early protein 6 functioning	Eversa et al. (82)
6	3-Methylquercetin	Effect on methylquercetin on poliovirus replication	Poliovirus	Blocks viral replication, selective inhibition of poliovirus RNA	Castrillo and Carrasco (83)
7	Miscellaneous phenolic compounds: Anthraquinone Chrysophanic acid Caffic acid Eugenin Hypericin Tannins (condensed polymers) Proanthocyanidins Salicylates Quinones Naphthoquinones Naphthoquinones Anthraquinones, in particular *Aloe emodin*	Effect of polyphenolics on viral inhibition		Viral RNA and DNA replication cycle interference	Takechi and Tanaka (84) Sydiskis et al. (85) Kurokawa et al. (86) Liu et al. (54)

8	Quercetin (Q) Luteolin (LU) 3-*O*-methylquercetin (3MQ)	Effect on the viral replication cycle of HSV-1	HSV-1	Interference with the events HSV-1 which includes transcription and translation of viral proteins	Bettega et al. (87)
9	Amentoflavone Scutellarein Quercetin	Study of effect on DNA synthesis	AMV (RAV-2) MMLV	Inhibit three reverse transcriptases (RT): AMV RT RAV-2 RT MMLV RT	Spedding et al. (88)
10	3(2H)-isoflavene	Action of the antiviral compound 3(2H)-isoflavene against Sabin type 2 poliovirus	Sabin Type 2 Poliovirus	3(2H)-isoflavene acts as a potent inhibitor of PV2 uncoating and targets the VP1 protein	Salvati et al. (89)
11	(−)EGCG (−) ECG (−) EGC	Effect on viral synthesis	Influenza virus	Suppression of viral RNA with EGCG and ECG whereas EGC failed to show similar effect	Song et al. (90)
12	Flavonoids complex:Amentoflavone Theaflavin Iridoids Phenylpropanoid glycosides Agathisflavone Robustaflavone Rhusflavanone Succedaneflavanone Chrysosplenol C	Effect on viral replication	HIV	Blockage of RNA synthesis exhibited HIV-inhibitory activity	Lin et al. (91) Semple et al. (92) Yu et al. (93)

(continued)

Table 24.2
(continued)

	Name of the phytochemicals/class	*Details of Study*	*Virus*	*Mechanism*	*References*
	Morin Coumarins Galangin (3,5,7-trihydroxyflavone) Baicalin Quercetin Isoquercetin				
13	Terpenoids:Parthenolide Sesquiterpene Triterpenoids Moronic acid Ursolic acid Maslinic acid Saponin	Effect on HCV replication in a subgenomic RNA replicon assay system	HCV	Potentiates the interferon α-exerted anti-HCV effect	Hwang et al. (94)
14	Polysaccharides carrageenan	Effect of polysaccharides on viral replication	HSV-1	Inhibition of viral replication subsequent to viral internalization	González et al. (95)
15	Algal polysaccharide	Effect on the production of retroviruses (murine leukemia virus – MuLV) and cell transformation by murine sarcoma virus (MuSV-124) in cell culture	Murine leukemia virus – MuLV Murine sarcoma virus (MuSV-124)	Action against the subsequent secondary infection cycle	Talyshinsky et al. (96)

16	Alkaloids	Mechanism of action of michellamine B Effects of Amaryllidaceae alkaloids/derivatives upon herpes simplex virus (type 1)	HIV Herpes simplex virus (type 1)	Michellamine B inhibits RT (early HIV life cycle) inhibiting cellular fusion and syncytium formation later Blocking of viral DNA polymerase activity	McMahon et al. (97) Renard-Nozaki et al. (98)
17	Lignans Nordihydroguaiaretic acid (NDGA (a lignan present in the perspired resin of leaves of *Larrea divaricata*)) Podophyllotoxin and related lignans (cyclolignanolides) such as the peltatins Dibenzocyclooctadiene lignans such as Schizarin B and Taiwanschirin D Rhinacanthin E and Rhinacanthin F	Effect on HBV, Influenza virus type A infection	HBV, influenza virus type A	Viral replication cycle inhibition	Konigheim et al. (61)
18	Olomoucine and roscovitine	Potential applications of CKIs are being studied presently in viral diseases	Cytomegalovirus and herpes simplex virus	Cyclin dependant kinase inhibitor inhibits viral replication	Bresnahan et al. (99)Schang et al. (100)
19	Psoralen compounds 4′-hydroxymethyltrioxsalen 4′-aminomethyltrioxsalen	Effect on infectivity of DNA and RNA virus (radioimmunoassay immunofluorescence)	Herpes simplex virus	Binds to nucleic acids when irradiated with long wavelength of UV light	Redfield et al. (101)

(continued)

Table 24.2
(continued)

	Name of the phytochemicals/class	*Details of Study*	*Virus*	*Mechanism*	*References*
20	DNJ	Action mechanism against bovine viral diarrhea virus	Bovine viral diarrhea virus	Reduction of viral secretion due to an impairment of viral morphogenesis (ER-glucosidase inhibition)	Durantel et al. (102)
21	Naturally occurring thiophene Alpha-terthienyl (1) 15 synthetic analogs	Photoactivated antiviral and cytotoxic activities against murine cytomegalovirus and sindbis virus, and murine mastocytoma cells	Murine cytomegalovirus sindbis virus	After irradiation with near UV light, alpha-terthienyl and most of its analogs had significant toxicity, with minimum inhibitory concentrations in the range of 0.02–40 μM	Marle et al. (103)
22	Gingerol	Common cold throat infection	Common cold virus	Improvement of NK cell lysing activity positive effect on immune system	Chrubasik et al. (74)
23	Capsaicin	HSV infections	HSV	Interference with intraneuronal transport of virus	Stanberry et al. (104)
24	Curcumin	Coxsackievirus infection	Coxsackievirus	Dysregulation of the UPS	Xiaoning et al. (105)
25	Lutein/zeaxanthin	HIV	HIV	Lowers oxidative stress/ metabolism restoration	Dikici et al. (106)

Table 24.3
Anti HIV activity of phytochemicals

Compounds	*Experiment details*	*Mechanism*	*Reference*
Carnosic acid	Displayed the strongest inhibitory effect (IC 90 = 0.08 μg/mL)	Protease inhibitors	Paris et al. (108)
Isoobtusitin	Effect on HIV replication	Interferes replication of the HIV virus	Chang et al. (109)
Tannins	Potent inhibitory activity	interferes HIV-1 replication, HIV-1-mediated cell fusion, and the gp41 six-helix bundle formation	Fortin et al. (110)
Compounds from Mulberry juice	Anti HIV	Anti-stress and anti-HIV activity evaluation in different fractions of the juice	Sakagami et al. (111)
HIV alkaloid drymaritin	Anti HIV	Anti-HIV effect in H9 lymphocytes with EC50 value of 0.699 μg/mL	Hsieh et al. (112)
7-*O*-β-d-(4″-caffeoyl) glucuronide	Anti-HIV activity in a cell culture assay (EC 50 = 41.86 ± 1.43 μg/mL)	Integrase inhibitory activity (IC50 = 7.2 ± 3.4 μg/mL)	Lee et al. (113)
Ferulic acid, gallic, caffeic, furulate, gallate, curcumin	Effect on replication	Replication inhibition	Olivero-Verbal and Pacheco-Londono (114)
Scutellarein and 6-hydroxyluteolin	Antiviral effect	Displayed strong HIV reverse transcriptase inhibition	Nishibe et al. (115)
Glycoside gallate ester	Antiviral effect		Kim et al. (116)
Chrysin, apigenin (**63**) and acacetin (**64**)	Antiviral effect	Interferes with HIV activation	Critchfield et al. (117)
Flavones and flavanones	HIV-1, HIV-2 or simian immunodeficiency virus	Binding of sCD4 and antibody to gp120	Mahmood et al. (118)
1,2,5,8-tetrahydroanthraquinone and hypericin	Antiviral effect	Found to inhibit HIV-1 reverse transcriptase	Schinazi et al. (119)

polio virus. Isoobtusitin (**61**), a prenylated coumarin showed substantial *in vitro* inhibitory activity against poliovirus (IC50 = 2.9 μM) (119). Isokaempferide (5,7,4′-trihydroxy 3-methoxyflavone) derived from *Psiadia* species was found to be an inhibitor of poliovirus type 2 replication (120). Tuli and colleagues examined the antiviral action of 3-methyleneoxindole (MO), a plant metabolite, in HeLa cells infected with poliovirus. On the basis of the experiments, authors suggested that the ability of MO to bind to ribosomes of HeLa cells may underlie the antiviral affect. Experiments showed that the poliovirus messenger RNA would not attach to those ribosomes that are already bound to MO. This resulted in the nonrecovery of virus-specific polysomes from infected cells treated with antiviral concentrations of MO (121) (Fig. 24.6).

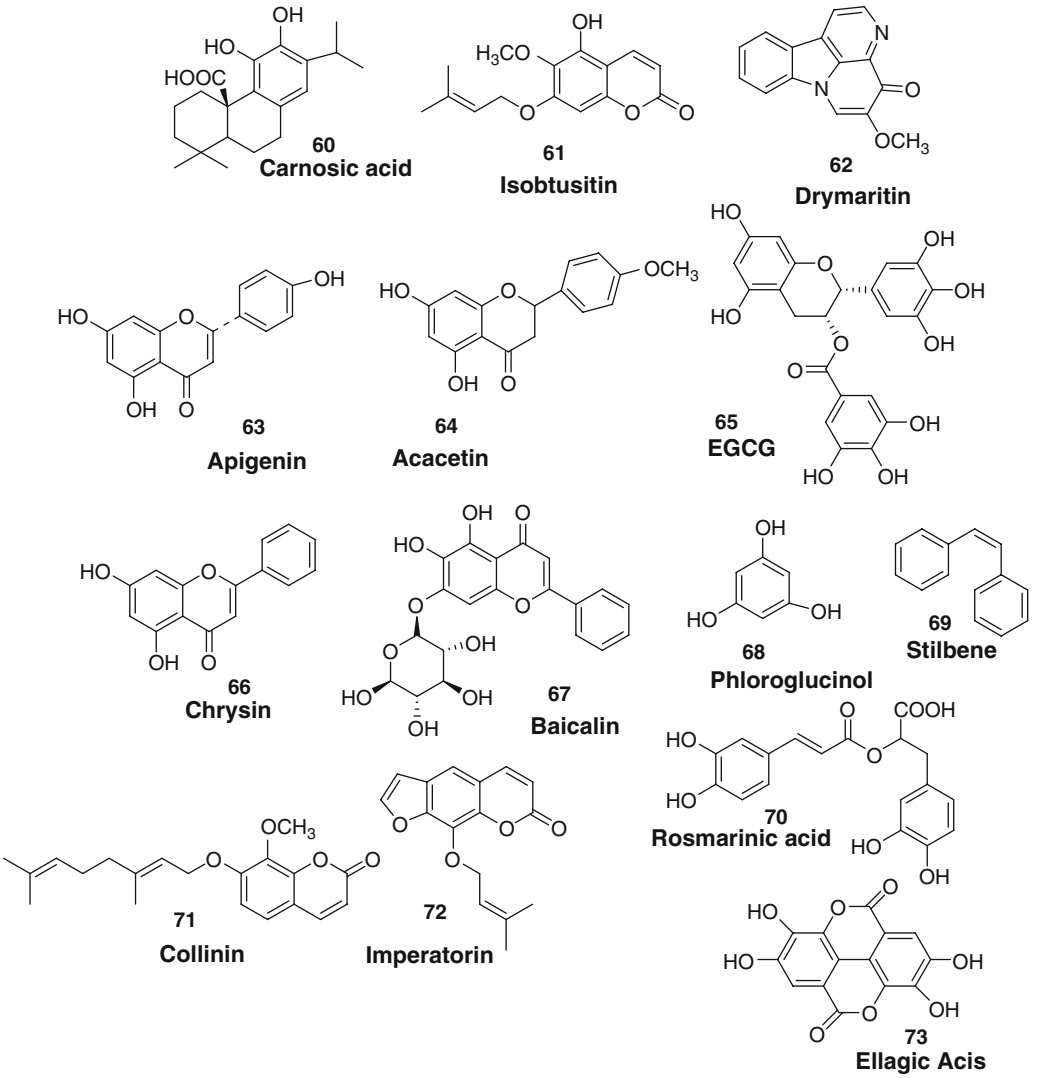

Fig. 24.6.

24.2.14 Herpes

Herpes is caused by HSV-1 and -2. It is a painful infection mainly affecting skin, eyes, mouth, and genitals. There is no permanent cure for herpes but the treatment can certainly reduce the viral shedding. There have been efforts all around the globe to identify plant-based treatment for this infection. Lyu et al. performed anti-herpetic assays on 18 flavonoids in five classes and a virus-induced cytopathic effect (CPE) inhibitory assay, plaque reduction assay, along with yield reduction assay (122). EC, ECG, galangin, and kaempferol exhibited strong antiviral activity whereas catechin, EGC, EGCG (**65**), chrysin (**66**), BA (**67**) showed moderate activity against HSV-1. Among all the flavanols, it was found that EC and ECG displayed a high level of CPE inhibitory activity (2.5 μM [0.725 μg/mL]) and 5 μM (2.21 μg/mL), respectively), while among the flavanones naringenin expressed a strong inhibitory effect (5 μM [1.36 μg/mL]) against HSV-1. Similarly, among the flavonols, quercetin exhibited a high CPE inhibitory activity (5 μM [1.69 μg/mL]), and genistein which is an isoflavone also showed an inhibitory effect (5 μM [1.35 μg/mL]). Two dibenzocyclooctane lignans, Kadsulignan L, and Neokadsuranin were tested for their anti-HBV activities *in vitro*. These compounds at 0.1 mg/mL, exhibited moderate antiviral activities, inhibiting HBsAg and HBeAg secretions by 32.6 and 36.5%, and by 14.5, and 20.2%, respectively. From a structure-activity point of view, it was found that the introduction of an a-orientated AcO group enhances the antiviral activity (123). Chattopadhyay and colleagues reported substantial anti-HSV activity of *Ophirrhiza nicobarica* extract at 300 μg/mL. The alkaloid, flavonoid, and β-sitosterol isolated from bioactive parts had a dose-dependent therapeutic efficacy, justifying their use (124). Eugenol (4-allyl-1-hydroxy-2-methoxybenzene) was screened for efficacy against HSV-1 and HSV-2 viruses. The *in vitro* experiments revealed that the replication of HSV viruses was inhibited by eugenol. The inhibitory concentration 50% values for the anti-HSV effects of eugenol were 25.6 μg/mL and 16.2 μg/mL for HSV-1 and HSV-2, respectively, with 250 μg/mL being the maximum dose at which cytotoxicity was tested. In addition, it's worth mentioning that eugenol showed no cytotoxicity at the concentrations tested. Furthermore, the eugenol–acyclovir combinations have synergistically inhibited herpesvirus replication *in vitro* (125). Nineteen compounds isolated from *Ranunculus sieboldii* and *Ranunculus sceleratus* were tested for inhibitory effects on hepatitis B virus (HBV) and HSV-1. The experiments revealed that apigenin 4′-*O*-α-rhamnopyranoside, apigenin 7-*O*-β-glucopyranosyl-4′-*O*-α-rhamnopyranoside, tricin 7-*O*-β-glucopyranoside, tricin, and isoscopoletin (**18**) possessed excellent antiviral activity against HBV replication. In addition, protocatechuyl aldehyde (**19**) also displayed substantial inhibiting activity on HSV-1 replication (126). Likhitwitayawuid et al. tested flavonoids, coumarins, phloroglucinol (**68**), and stilbenes (**69**) derivatives derived from *Mallotus pallidus*, *Artocarpus gomezianin,* and *Triphasia trifolia*. It was concluded that bis hydroxyphenyl structures are promising candidates for anti-HSV and anti-HIV drug development (127). The *in vitro* antiviral activity of galangin (3,5,7-trihydroxyflavone), the major antimicrobial compound isolated from the shoots of *Helichrysum aureonitens*, was investigated against herpes simplex virus type 1. The compound showed significant antiviral activity against HSV-1 (an enveloped double-stranded DNA virus) and Cox B1 (an un enveloped single-stranded RNA virus) at concentrations varying from 12 to 47 μg/mL (128).

Epigallocatechin 3-*O*-gallate, samarangenin B derived from the roots of *Limonium sinense* had higher inhibitory activity than the positive control acyclovir. All of these were examined for inhibitory effect against the replication of HSV-1 virus in Vero cells (129). Du et al. isolated flavonoid leachianone from the root bark of *Morus alba* showing potent antiviral activity. A flavonoid moralbanone, having characteristic prenyl chain, along with seven other known compounds, was isolated from the root bark of *Morus alba* L. Among all the isolated compounds, Leachianone G showed potent antiviral activity (IC50 = 1.6 μg/mL) (130). Three new flavonol glycosides, namely, isorhamnetin 3-*O*-(6″-*O*-(Z)-p-coumaroyl)-β-d-glucopyranoside, quercetin 3-*O*-α-l-rhamnopyranosyl(1-2)-α-L-arabinopyranosyl(1-2)-α-L-rhamnopyranoside, and quercetin 3-*O*-α-L-arabinopyranosyl(1-2)-α-L-rhamnopyranoside, were isolated from the stems of *Alphitonia philippinensis* collected from Hainan Island, China. Some of the isolated triterpenoids and flavonoid glycosides showed cytotoxicity against human PC-3 cells and hepatoma HA22T cells, and the inhibition of replication on HSV-1 (131). Viral diseases, especially of skin, can be treated with a virucide encapsulated in multilamellar phospholipid liposomes. Rosmarinic acid (**70**), incorporated in phospholipid mixture demonstrated effectiveness in humans afflicted with HSV (132). Flavonol glycosides (from quercetin and isorhamnetin) derived from the stems of *Alphitonia philippinensis* have been reported to inhibit the replication of HSV-1. Isodihydrosyringetin, a new (2R,3S)-3,5,7,4′-tetrahydroxy-3′,5′-dimethoxyflavanone was extracted from the root of *Limonium sinense* (Girard) along together with nine other known compounds. Out of all the compounds examined for their inhibitory effects on HSV-1, replication in vero cells, epigallocatechin 3-*O*-gallate and samarangenin B exhibited potent inhibitory activities on HSV-1 replication. Comparison of the IC50 values indicated that these both compounds had higher inhibitory activities than the positive control acyclovir (38.6 ± 2.6 vs. 55.4 ± 5.3 μM, $P < 0.001$; 11.4 ± 0.9 vs.55.4 ± 5.3 μM, $P < 0.0005$) (129). *Cedrus libani*, widely used as traditional medicine in the middle east for the treatment of different infections was studied for its antiviral potential. The phytochemical components isolated himachalol (22.50%), β-himachalene (21.90%), and α-himachalene (10.50%) showed promising results against herpes simplex virus type 1 (HSV-1) (133). Harden et al. evaluated the antiviral activity of extracts from *Undaria pinnatifida*, *Splachnidium rugosum*, *Gigartina atropurpurea*, and *Plocamium cartilagineum* against HSV-1 and HSV-2. Different assays showed that the compounds had potent virucidal activity and were active at very low concentrations (134). There are already reports in literature regarding excellent anti-HSV activity of *Maclura cochinchinensis* in several *in vitro* experiments. The authors have carried out biologically-guided separation of the active component(s). Ethyl acetate and methanol extracts exhibited anti-HSV-2 activity at EC50 values of 38.5 μg/mL and 50.8 μg/mL, respectively. Biologically-guided chromatographic separation of the ethyl acetate extract yielded compound A, identified as morin using a spectroscopic method. Morin exhibited anti-HSV-2 activity at an EC50 value of 53.5 μg/mL. In order to test the activity of acetate derivative, morin penta acetate was synthesized; however, the compound did not show any activity. It was concluded that free hydroxyl groups were required for anti-HSV-activity, as demonstrated previously by other workers for the antiviral activity of other flavonoids (135).

24.2.15 Hepatitis

Hepatitis derives its name from the Greek words *hepato* and *itis* which literally stands for liver inflammation. There are several types of viral Hepatitis such as Hepatitis A, B,C,D,E,F,G. Hepatitis is also caused by mumps virus, rubella virus, and cytomegalovirus. A large number of herbal products have been screened to measure their efficacy as anti-hepatitis drugs. One of the coumarin derivative geranyloxy-8-methoxycoumarin, best known as collinin (**71**) obtained from *Zanthoxylum schinifolium* was shown to significantly inhibit the replication of hepatitis B virus DNA (IC50 = 17.1 µg/mL (109). Seven plant extracts from six different families were found to have antiviral activity against HSV-1, at a concentration non toxic to the cell line (Vero) used. It was shown that most of these extracts have partial activity at the low concentration used. The methanol extracts of the aerial parts of *Hypericum mysorense* and *Hypericum hookerianum*, exhibited detectable antiviral effect towards HSV-1 with an inhibitory concentration for 50% (IC^sub 50^) of 100 and 50 µg/mL respectively (135). The administration of concanavalin A (Con A) to mice induces cytokine-dependent hepatitis. Okamoto et al. examined the effect of glycyrrhizin on Con A-induced hepatitis and showed that glycyrrhizin inhibited Con A-induced hepatitis without affecting cytokine expression (136) (Fig. 24.7).

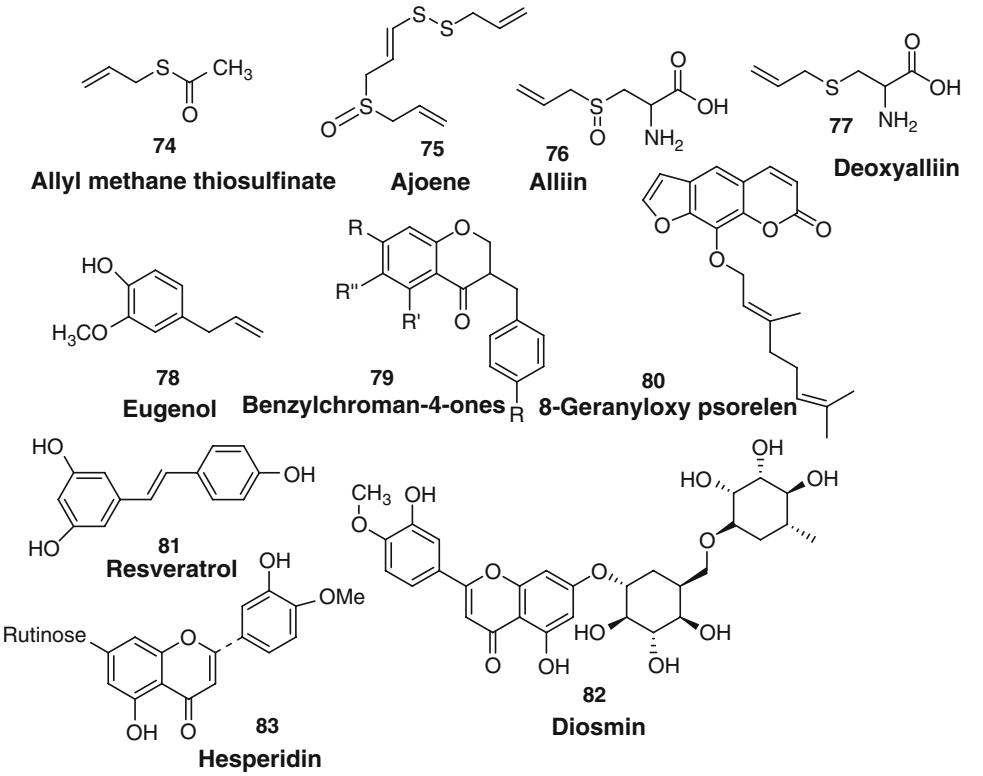

Fig. 24.7.

Constituents isolated from *Ranunculus sieboldii* and *Ranunculus sceleratus* were tested for inhibitory effects on hepatitis B virus (HBV) and HSV-1. It was shown that apigenin 4′-*O*-α-rhamnopyranoside, apigenin 7-*O*-β-glucopyranosyl-4′-*O*-α-rhamnopyranoside, tricin 7-*O*-β-glucopyranoside, tricin, and isoscopoletin possessed substantial inhibitory activity against HBV replication (126). Ellagic acid (**73**), isolated from *Phyllanthus urinaria,* has exhibited the blockage of HBeAg secretion in HepG2 2.2.15 cells. Since HBeAg is involved in immune tolerance during HBV infection, ellagic acid may be a new therapeutic candidate against immune tolerance in HBV-infected individuals (137).

24.2.16 Influenza

Influenza virus (an RNA virus belonging to the *Orthomyxoviridae* family) is the causative organism for influenza commonly known as viral flu. There are three types of viruses known to cause influenza: influenza virus A, B and C. As an integral part of traditional therapy in India and China, plant extracts have been routinely used to cure flu since old times. A number of active biological compounds have been found to possess excellent antiviral activity against the influenza virus. Antiviral flavonoid 2″-*O*-(2‴-methylbutanoyl) isoswertisin obtained from the flower of *Trollius chinensis* was found to be moderately active toward influenza virus A. Two new flavonoid-type C-glycosides, trollisin I (=(1S)-1,5-anhydro-1-[2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxy-4-oxo-4H-[1]benzopyran-8-yl]-2-*O*-(2-methylbutanoyl)-d-glucitol) and its 2-*O*-benzoyl congener trollisin II, were isolated from *Trollius chinensis*, together with the two known compounds 2″-*O*-(2‴-methylbutanoyl) isoswertisin and vitexin galactoside. In antiviral assays, the compounds were found to be moderately active towards influenza virus A (138). The inhibiting effects of isoscutellarein-8-methylether (5,7,4′-trihydroxy-8-methoxyflavone, F36) obtained from *Scutellaria baicalensis* on the single-cycle replication of mouse-adapted influenza viruses A/Guizhou/54/89 (H3N2 subtype) and B/Ibaraki/2/85 was evaluated and it was reported that the flavone significantly suppressed the replication of these viruses in a dose-dependent manner. It was noticed that the agents suppressed the replication of these viruses from 6 to 12 h after incubation in a dose-dependent manner by 50% at 20 μM and 90% at 40 μM, respectively. Remarkably 5,7,4′-trihydroxy-8-methoxyflavone, at the concentration of (50 μM) reduced the release of B/Ibaraki virus in the medium by 90–93% when it was added to the MDCK cells at 0–4 h after incubation (139). In a series of experiments, the phenolic biopolymer SP-303 was tested for its efficacy against experimentally induced influenza A (H1N1) virus infections in mice. It was found that when 30, 10, or 3 mg/kg/day of SP-303 was administered intraperitoneally once daily for 8 days, beginning either 48 h before or 4 h after virus exposure, only lung consolidation was significantly reduced (140).

24.2.17 Common Cold

Common cold is caused by Rhinovirus, (derived from the Greek word rhin- denoting nose) belonging to the *Picornaviridae* family. Traditional forms of medicines have relied on plant preparations to cure common cold, especially in the Indian subcontinent. Employing a plaque reduction assay, several homo-isoflavonoids and chloro-substituted

rac-3-benzylchroman-4-ones were evaluated for *in vitro* activity against selected picornaviruses. All homo isoflavonoids that were tested exhibited an inhibitory effect on rhinovirus replication with an activity depending on virus serotype and compound (141). Douglas and colleagues have reported antiviral activity of Vitamin C against rhinovirus (142). In another report, plants derived from the *Echinacea* family (family *Asteraceae*) have been shown useful for preventing and treating the common cold (143). The antiviral activity of different 2-styrylchromones was evaluated and almost all of them displayed activity against serotypes of human rhinovirus, 1B in a plaque reduction assay in HeLa cell cultures. Mechanistically, the compounds were found to interfere with HRV 1B replication. The antiviral activity of 2-styrylchromones and 3-hydroxy-1-(2-hydroxyphenyl)-5-phenyl-2,4-pentadien-1-ones, which are intermediates in the synthesis have been evaluated against two selected serotypes of human rhinovirus, 1B and 14, by a plaque reduction assay in HeLa cell cultures. It was found that al most all the compounds interfered with HRV 1B replication, with the exception of 3-hydroxy-1-(2-hydroxyphenyl)-5-(4-methoxyphenyl)-2,4-pentadien-1-one which did not show any significant activity. It is worth mentioning that the majority of derivatives were found to be effective against serotype 14, often with a higher potency (144, 145).

24.2.18 Multiple Targets

A considerably large number of studies has reported the activity of various phytochemicals against multiple targets. Weber et al. used direct pre-infection incubation assays to determine the *in vitro* virucidal effects of fresh garlic extract, its polar fraction, and other garlic-associated compounds, that is, diallyl disulfide (**37**), diallyl thiosulfinate (**39**) (allicin), allyl Me thiosulfinate (**74**), ajoene (**75**), alliin (**76**), deoxyalliin (**77**), and diallyl trisulfide (146).

In an effort to determine the mechanistic action of garlic compounds to explain their antiviral action, direct pre-infection incubation assays were used to determine the *in vitro* virucidal effects against selected viruses including, HSV-1, HSV-2, Para influenza virus type 3, vaccinia virus, vesicular steatites virus, and human rhinovirus type 2. These results indicate that virucidal activity and cytotoxicity may have depended upon the viral envelope and cell membrane, respectively. However, activity against non-enveloped virus may have been due to the inhibition of viral adsorption or penetration. The order for virucidal activity generally was: ajoene (**66**) > allicin (**39**) > allyl Me thiosulfinate (**74**). Tait et al. showed marked antiviral activity of homoisoflavonoids against coxsackie virus B1, B3, B4, A9, and echovirus 30. The inhibition of viral replication was monitored on BGM cells. Out of the various tested compounds, 3-benzyl chroman-4-ones (**79**) have displayed substantial antiviral effect towards PI-3 (parainfluenza-3) in the range of 8–32 µg/mL of inhibitory concentration for cytopathogenic effect (CPE) in Madin–Darby bovine kidney and vero cell lines (146) Eugenol, (**78**) a traditional medicine has also been used against multiple viral targets (125). Singh et al. have investigated the interaction between chemokine receptor CXCR4 and flavonoids using in silico docking studies. On the basis of their studies, the authors concluded that flavonoids may also be useful as topical agents to inactivate virus, or may act as adjuvant with other antiviral drugs. Interaction network formed by disulfide bonds, hydrogen bonds, van der Waals force, and salt bridges between extracellular segments helped in maintaining the conformation

of the docked complex (147). The moderate antiviral activity of the mixture of quercetin 3-*O*-β-glucoside and quercetin 3-*O*-β-galactoside derived from *Chamaesyce thymifolia* against HSV-1 and BVDV viruses was also reported (148). A number of substituted homo-isoflavonoids were synthesized in order to study their *in vitro* anti-picornavirus activity. Experiments were performed to determine the ability of non-cytotoxic concentrations to interfere with plaque formation by HRV 1B and 14 and poliovirus (PV) 2. Experiments suggested that serotype 1B was much more sensitive than 14 to the action of the compounds, and the presence of one or more chlorine atoms increased the antiviral effect in all homo isoflavonoids tested, confirming the positive influence of this substituent on activity (149). In an attempt to search for novel active agents from plant source pure flavonoids and aqueous extracts of *Caesalpinia pulcherrima* Swartz were screened to test their influence on a series of viruses, namely HSV-1, HSV-2, and adenoviruses (ADV-3, ADV-8, ADV-11). Results showed that the aqueous extracts of *C. pulcherrima* and its related quercetin possessed a broad-spectrum antiviral activity. The experiments have shown that fruit and seed extract showed the best activity (EC50 = 41.2 mg/L, SI = 83.2) as compared to stem and leaf (EC50 = 61.8 mg/L, SI = 52.1) and flower (EC50 = 177.9 mg/L, SI = 15.5). Quercetin derived from the plant possessed the strongest anti-ADV-3 activity (EC50 = 24.3 mg/L, SI = 20.4) (150). In the last decade, there has been a lot of focus on the amino sugar glucosidase inhibitors have selective antiviral activity against certain enveloped, mammalian viruses (151). It has been shown that deoxynojirimycins (DNJs) modified by reductive amination to attach a long chain to N atom (their N-DNJ derivative) were shown to be, for example, at least 20 times more potent than the non-alkylated DNJ in inhibiting hepatitis B virus (HBV) and bovine viral diarrhea virus (BVDV) in cell based assays. These data suggested that the modification of the alkyl side chain could influence antiviral activity (152). De Almeida et al. reported strong inhibition of an infusion of *Persea americana* leaves against HSV-1, Aujeszky's disease virus (ADV) and adenovirus type 3 (AD3) in cell cultures. An extract of *Persea americana* leaves (Lauraceae) strongly inhibited herpes simplex virus type 1 (HSV-1), Aujeszky's disease virus (ADV) and adenovirus type 3 (AD3) in cell cultures. Its fractionation, guided by anti-HSV-1 and ADV assays, allowed the isolation and identification of two new flavonol monoglycosides, kaempferol and quercetin 3-*O*-α-d-arabinopyranosides, along with the known kaempferol 3-*O*-α-l-rhamnopyranoside (afzelin), quercetin 3-*O*-α-l-rhamnopyranoside (quercitrin), quercetin 3-*O*-β-glucopyranoside and quercetin. In the extract, the known quercetin 3-*O*-β-galactopyranoside was also identified. The authors have reported that afzelin and quercetin 3-*O*-α-d-arabinopyranoside showed higher activity against acyclovir-resistant HSV-1. Chlorogenic acid significantly inhibited the HSV-1 replication without any cytotoxicity. However, all the substances tested were less active than the infusion or fractions (153). A summary of major classes of antiviral phytochemicals along with their source and viral targets has been provided in Table 24.4.

24.2.19 Miscellaneous

There exists a huge volume of literature regarding the evaluation of plant-derived compounds against several other viral targets apart from the ones listed above. Substantial antiviral activity of 8-geranyloxypsoralen (**80**) (isolated in low yields from

Table 24.4
Major classes of antiviral phytochemicals

Compound class	*Virus type*	*Name of the plants*	*References*
Polyphenols	HSV-1	*Agrimonia pilosa*, *Punica granatum*, *Moringa oleifera*, *Aglaia odorata*, *Ventilago enticulata*	Li et al. (154)
Torvanol		*Solanum torvum*	Lipipun et al. (155)
Flavonoids		*Morus alba*	Arthan et al. (156)
Saponins		*Maesa lanceolata*	Du et al. (130)
Anthraquinone, flavone		*Rheum officinale*, *Aloe barbadensis*, *Cassia augustifolia*	Aspers et al (157)
Essential oil		*Santalum album*, *lemon grass*	Sydiskis et al. (158)
Essential oil		*Artemisia douglasiana*, *Eupatorium patens*, *Tessaria absinthioides*	Jassim and Naji (159)
Meliacine (peptide)		*Melia azedarach*	Garcia et al. (160)
Saponin		*Bupleurum rigidum*	Alche et al. (161)
Rosmarinic acid		*Aloe emodin*	Baermejo et al. (162)
Oils		*Minthostachys verticillata*	Sydiskis et al. (158)
			Primo et al. (163)
Essential oil	HSV-1 and HSV-2	*Eupatorium patens*	Garcia et al. (160)
Eugeniin (tannin)		*Geum japonicum*	Khan et al. (164)
Ursolic acid		*Alstonia macrophylla*	Chattopadhyay et al. (165)
Morin (triterpene)	HSV-2	*Rus javanica*	Khan et al. (164)
Casuarinin (tannin)		*Terminalia arjuna*	Cheng et al. (166)
Oils		*Melissa officinalis*	Allahveridev et al. (167)
Tannins	Measles	*Bambusa vulgaris*	Ojo et al. (168)
Phenolic compounds	Yellow fever Polio	*Aframomum melegueta*	
Rosmarinic chlorogenic caffeic acids (phenolics)	VZV influenza, PRV	*Aloe emodin*, *Aloe barbadensis*	Sydiskis et al. (158)
Essential oil	HSV, ADV 8	*Boussingaultia gracilis*, *Serissa japonica*	Chiang et al. (169)
Essential oil	Dengue-2	*Artemisia douglasiana*, *Eupatorium patens*	Garcia et al. (160)

(continued)

Table 24.4
(continued)

Compound class	*Virus type*	*Name of the plants*	*References*
Oils	Psuedorabies	*Minthostachys verticillata*	Primo et al. (163)
Flavonoids	Influenza A	*Barleria prionitisBlumea laciniata*, *Markhamia lutea*,	Choi et al. (170)
Polyphenols	RSV	*Elephantos scaber*, *Mussaenda pubescens*, *Scutellaria indica*	Kernan et al. (171) Li et al. (81)
Flavonoids	RSV, influenza	*Aesculus chinensis*	Wei et al. (172)
Tannins polyphenols	Influenza	*Bergenia ligulataGeranium sanguineum*	Rajbhandri et al. (173) Sokmen et al. (174)
Diterpenoid	Para influenza 3	*Caesalpinia minax*	Jassim and Naji (159)
Alkaloid	Measles	*Zanthoxylum chalybeum*	Olila et al. (175)
Essential oil	HBV	*Rheum palmatumPhyllanthus niruri*,	Jassim and Naji (159)
Chebulic acid (tannin)		*Phyllanthus urinaria*	Thyagarajan et al. (176)
Niruriside		*Phyllanthus* spp.	Thyagarajan et al. (177)
		Sophorae flavescentis	Liu et al. (178)
Flavonoids	HCV	*Amebia*, *euchroma*, *Thalaspi arvens*, *Poncirus trifoliata*	Ho et al. (179)
Flavonoids	HCV	*Glycyrrhizae radix*	Sekine et al (180)
Usnic acid	HIV	Lichen *Ramalina farinacea*	Esimone et al. (181)
Triterpenoid		*Brazilian propolis*	Manfredi et al. (182)
Flavonoid		*Glycyrrhiza lepidota*, *G. glabra*	Manfredi et al. (182)
Saponin		*Maesa lanceolata*	Apers et al. (157)
Flavone		*Desmos* spp.	Wu et al. (183)
Flavonoids		*Alianthus altissima*	Chang et al. (184)
Flavonoids		*Begonia nantoensis*	Wei et al. (172)
Specific lectin		*Momordica charantia* L.	Cos et al. (72)
Proteins (RIP), mannose specific lectins		*Cymbidium* spp.	Balzarini et al. (185)
		Urtica dioca	Yogeeswari and Sriram (186)
GAP-31 lectins			Jay et al. (187)
Lectin			

Saponin	HIV entry	*Tieghemella heckelii*	Gosse et al. (188)
Alkaloid	HIV-1	*Stephania cepharantha*	Ma et al. (189)
Coumarin		*Prangos tschimganica*	Shikishima et al. (190)
Triterpenoids		*Vatica cinerea*	Zhang et al. (191)
Alkaloids		*Leucojum vernum*	Szlavik et al. (192)
Flavonoid	HIV replication	*Scutellaria baicalensis*	Li et al. (81)
Polypeptides	HIV-1 RT	*Phaseolus vulgaris* and *Phaseolus coccineus*	Ye and Ng (193)
Coumarins		*Callophyllum lanigerum*	Cos et al. (72)
Flavonoids		*Dryopteris crasissirhizoma*	Min et al. (194)
Polypeptides		*Momordica charantia*	Jiratchariyakul et al. (195)
Protein		*Panax notoginseng*	Ye and Ng (193)
Tannin		*Shepherdia argentia*	Notka et al. (196)
Gallotannins		*Phyllanthus* spp.	Notka et al. (196)
Ursolic acid (triterpene)	HIV-1 protease	*Geum japonicum*	Park et al. (197)
Camelliatannin (tannin)		*Camellia japonica*	
Polyphenol	HIV fusion	*Prunella vulgaris*	Liu et al. (198)
Tannin		*Rhizoma cibolte*	
Curcumin (phenolics)	HIV integrase and protease	*Curcuma linga* L., *Larrea tridentata* L.	Cos et al. (70)
Lignan			
Phorbol ester	HIV replication	*Homolanthus mutans*	Yogeeswari and Sriram (186)
Phorbol ester	HIV gene expression	*Euphorbia poissonii*	Yogeeswari and Sriram (186)
Epigallocatechin-3-gallate	Epstein–Barr virus	*Green tea*	Choi et al. (199)
Kaempferol	Poliovirus 2,3	*Dianella longifolia*, *Pterocaulus sphaedatum*	Andres et al. (200)
Anthraquinone		*Psiadia dentata*	Semple et al. (92)
Kaempferol			Robin et al. (201)
Oils	Junin virus	*Lippia junelliana*, *Lippia turbinata*, *Heterotheca latifolia*, *Tessaria absinthides*	Garcia et al. (160)
Tannin	Epstein–Barr virus	*Syzygium aromaticum*	Jassim and Naji (159)
Oligophenols	HCV protease	*Stylogne cauliflora*	Hegde et al. (202)
Theaflavin catechin flavonoid	Rotavirus, coronavirus	*Camellia sinensis*, *Eleutherococcus senticosus*	Clark et al. (203) Turan et al. (204)

Citrus limon) was reported against tumor promoter TPA-induced Epstein–Barr virus activation (10 μM, the inhibitory activity was 79.3%) (111). Well-studied polyphenol Resveratrol (**81**) was found to inhibit varicella–zoster virus (VZV) replication in a dose-dependent and reversible manner. RT-PCR studies showed that protein and mRNA levels of IE62, an essential early viral protein, were reduced when compared to controls (205). Baicalin (BA) derived from *Scutellaria baicalensis* has shown substantial antiviral activities. Mechanistically, it was shown that BA inhibited the binding of a number of chemokines to human leukocytes or cells transfected to express specific chemokine receptors (206).

Antiviral activities of seven compounds belonging to kaempferol family were evaluated against human HCMV and it was confirmed that the presence of acyl group is important for the activity (207). A freshly prepared extract of *Chelidonium majus* was tested *in vivo* for anti-retroviral activity using highly susceptible C57Bl/6 strain in a mouse. The mice were infected intraperitoneally with 0.2 mL of the stock virus pool of defective murine leukemia retroviruses (MuLVs) LP-BM5. The animals were sacrificed (after 4 months) and a significant reduction in the weight of spleen and cervical lymph nodes was noticed in chronically infected mice treated with freshly prepared crude extract of *Chelidonium majus* ($P = 0.0057$ and $P < 0.001$) (208). In an effort to elucidate the action mechanism of 3-methyl quercetagetin, it was reported that the significant activity of the compound against tomato bushy stunt virus was attributed to the interference during the virus infection initiation (209). Sanchez and colleagues evaluated the possible antiviral effect of flavonoids obtained from *Tephrosia madrensis*, *Tephrosia viridiflora,* and *Tephrosia crassifolia* on dengue viruses and concluded that glabranine and 7-*O*-methyl-glabranine presented 70% inhibition on the dengue virus (210). 4-hydroxypanduratin A and panduratin chalcone derivatives derived from *Boesenbergia rotunda* displayed substantial inhibitory activities toward dengue 2 virus NS3 protease (Ki values of 21 and 25 μM, respectively) (211). The inhibitory effects of diosmin (**82**) and hesperidin (**83**) on the infectivity of rotavirus causing sporadic diarrhea in infants was evaluated and it was shown that both compounds were effective against rotavirus infection (212). Some of the phytochemicals have graduated to the clinical trials. Owing to the space constraints only the major clinical trials relating to the antiviral activities of phytochemicals have been summarized in Table 24.5.

24.3 CONCLUSIONS AND PERSPECTIVES

Numerous epidemiological and experimental studies have revealed that a large number of the phytochemicals have promising antiviral activities. However, as discussed earlier, the development of new and better antiviral agents from plants pose a formidable challenge. One of the major challenges has been the relatively fewer number of *in vivo* studies coupled with inconsistency in results due to a lack of uniformity in the assays. Further, the data on the absorption metabolism and the excretion of phytochemicals in humans is contradictory and scarce. A highly interdisciplinary approach with meticulous planning and design needs to be followed for conducting the *in vivo* studies in a highly standardized environment. Consequently, the properly designed and rigorously executed clinical trial can help us to establish the efficacy and safety of the potential drug. In order to apply plant-based agents as an effective strategy, it is of

Table 24.5
Clinical trials

	Study details/condition treated	*Agent*	*Results*	*References*
1	Chronic hepatitis B	*Phyllanthus amarus*	Substantially cleared hepatitis B surface antigen	Thyagarajan et al. (176)
2	Chronic hepatitis B	*Phyllanthus amarus*	Significantly cleared hepatitis B surface antigen	Thyagarajan et al. (177)
3	Chronic hepatitis B	*Phyllanthus amarus*	No significant changes in levels of HbsAg	Thamlikitkul et al. (213)
4	Chronic hepatitis B	*Phyllanthus amarus*	No changes in levels of HBsAg, HBeAg, HBV DN	Berk et al. (214)
5	Chronic hepatitis B	*Phyllanthus amarus*	Significant reduction in HBeAg in treated group	Zhang et al. (215)
6	Chronic hepatitis B	*Phyllanthus amarus*	No significant changes in levels of HBsAg, HbeAg	Miln et al. (216)
7	Upper Respiratory Tract Infections (URTIs)	Chinese Herbal Medicine	Relieves external symptoms and effectively clear up the pathogenic cold	(217)
8	Chronic hepatitis B	*Phyllanthus amarus*	Significant reduction in HBeAg in treated group	Huang et al. (218)
9	Chronic hepatitis B	*Phyllanthus urinaria*	Significant reduction in HBeAg in treated group	Zhu et al. (219)
10	Chronic hepatitis B	*Phyllanthus urinaria*	Changes in levels of HBsAg, HBeAg, HBV DNA, HBV DNAP, significant reduction in HBsAg, HBV DNA, HBV DNAP in treated group	Cao et al. (220)
11	Chronic hepatitis B	*Phyllanthus urinaria*	Significant reduction in HBV DNA in treated group, improvement in liver function	Huang et al. (218)
12	Chronic hepatitis B	*Phyllanthus amarus*, *Phyllanthus niruri*, *Phyllanthus urinaria*	Improvement in patient condition *Phyllanthus urinaria* specifically effective, no side effect	Wang et al. (221)
13	Chronic hepatitis B Acute hepatitis B	*Glycyrrhiza glabra* *Glycyrrhiza glabra*	Changes in HBsAg, HBeAg, liver function, IgA, Ig G, IgM 50% acute and chronic cases cleared HBsAg and HBeAg in treated group vs. none of controls	Su et al. (222)
14	Chronic hepatitis B and C	*Glycyrrhiza glabra*	ALT levels in Group A significantly improved over levels in Group B ($P = 0.005$)	Iino et al. (223)

(continued)

Table 24.5
(continued)

	Study details/condition treated	*Agent*	*Results*	*References*
15	Hepatitis B virus	*Phyllanthus amarus*	Failed to inhibit B surface antigen in patient with hepatitis B virus	Doshi et al. (224)
16	Chronic hepatitis C	*Iscador* (*Viscum album extract*)	Substantial decrease in HCV production	Tusenius et al. (225)
17	Chronic hepatitis C	*Glycyrrhiza glabra*	Mean decrease in ALT levels 26% in treated group, 6% in placebo	van Rossum et al. (226)
18	Chronic hepatitis C	*Glycyrrhiza glabra*	Significantly greater reduction in all three parameters AST, ALT, GGT in Group B than in Group A	Tsubota et al. (227)
19	Hepatitis C	*Glycyrrhiza glabra*	Mean change in ALT levels 47%	van Rossum et al. (228)
20	Hepatitis C	*Phyllanthus niriri*	Healing complete by day 8 in 96% (natural recovery usually 10–14 days)	Mehrotra et al. (229)
21	Hepatitis C patients	Phlogenzym, a combination of hydrolytic enzymes with the flavonoid rutosid	Phlogenzym superior to ribavirin and interferon (established medication). The tolerance of the oral enzymes was excellent	Stauder and Kabil (230)
22	Chronic hepatitis	*Glycyrrhiza glabra*	Overall improvement in clinical markers and in some liver function tests	Suzuki et al. (231)
23	Chronic viral hepatitis	*Glycyrrhiza glabra*	Improvement ALT levels in Group A than Group B ($P = 0.0002$)	Miyake et al. (232)
24	Alcoholic or non-alcoholic chronic hepatitis	Silybin/phosphatidylcholine complex	Statistically significant drop ($P < 0.01$–0.001) in ALT and GGT occurred at doses of 240 mg or more	Vailaii et al. (233)
25	Jaundice in HBV persons	*Phyllanthus amarus*	No significant intergroup differences	Narendranathan et al. (234)
26	Liver cirrhosis	*Silymarin*	Silymarin has no effect on survival	Peres et al. (235)

27	Liver cirrhosis	*Silymarin*	Indicated effectiveness in patients with alcoholic cirrhosis	Ferenci et al. (236)
28	Influenza A	*Sambucus nigra* L.	Disappeared 4 days earlier in treated group	Thom et al. (237)
29	Influenza B	*Sambucus nigra* L.	Significantly faster recovery(treated group)	Zakay-Rones et al. (238)
30	Common cold	*Andrographis paniculata*	Visual analog scale, assessment of symptoms, days sick leave significant reduction in symptoms and in days sick leave in treated group	Melchior et al. (239)
31	Common cold	*Andrographis paniculata*	Significant reduction in intensity of symptoms in treated group	Caceres et al. (240)
32	Common cold	*Andrographis paniculata*	Reduced symptoms and faster recovery in treated group	Hancke et al. (241)
33	Common cold (prevention and treatment)	*Allium sativum* L.	Significantly fewer colds of shorter duration in treated group	Josling (242)
34	Rhinovirus infection	*Dichloroflavan* was given orally	Administration of dichloroflavan in the oral formulation tested is not of value in the treatment of human rhinovirus infection	Phillpotts et al. (243)
35	Chronic HCV patients	*Silymarin* capsules	Well tolerated patients improved over time	Tanamly et al. (244)
36	Chronic HCV	*Silymarin*	No visible effect	Gordon et al. (245)
37	Chronic HCV	*Silymarin* antioxidants and vitamins herbals	Well tolerated 48% patients positive response	Melham et al. (246)
38	HCV patients	*Silymarin*	No adverse effect but no effect on outcome	Strickland et al. (247)
39	Patients with detectable HCV RNA	*Silymarin* extract	Non interferon based standard therapy better than silymarin	El-Zayadi et al. (248)
40	HSV viral infection	*Melissa officinalis*	Treatment effective without any side cytotoxic side effects	Koytchev et al. (249)
41	Herpes simplex infection <72 h duration	*Melissa officinalis* L.	Significant reduction in symptom score in treated group on day 2	Wöbling et al. (250)

(continued)

Table 24.5
(continued)

	Study details/condition treated	*Agent*	*Results*	*References*
42	Genital herpes	*Aloe vera*	Mean days to healing, number of patients cured Significantly shorter mean time to healing in group (a) cured patient numbers greater in group (a) than group (b) or placebo	Syed et al. (251)
43	Genital herpes	*Aloe vera*	Mean days to healing, number of patients cured, shorter mean time to healing and cured patients at 2 weeks in treated group	Syed et al. (252)
44	Genital herpes	*Clinacanthus nutans*	*Phyllanthus niruri*, has anti-HBsAg activity	Jayavasu et al. (253)
45	Recurrent herpes labialis	*Melaleuca alternifolia*	Time to lesion healing No significant intergroup differences	Carson et al. (254)
46	Herpes labialis <24 h	*Salvia officinalis*	No significant intergroup differences	Saller et al. (255)
47	Herpes zoster	*Clinacanthus nutans*	Lesion healing significantly faster in treated group	Sangkitporn et al. (256)
48	Herpes zoster	*Clinacanthus nutans*	Complete healing, lesion healing significantly faster in treated group	Charuwichitra et al. (257)
49	HIV	*Andrographis paniculata*	Changes in levels of CD4 HIV-1 RNA Significant rise in CD4 Levels after 10 mg/kg, trial interrupted at 6 weeks due to adverse events	Calabrese et al. (258)
50	HIV	*Buxus sempervirens*	Significant delay of progression to disease	Durant et al. (259)
51	HIV	*Glycyrrhiza glabra*	Some improvement in asymptomatic carriers, none in AIDS patients	Gotoh et al. (260)

extreme importance to understand the molecular and cellular mechanism of the compounds with proper understanding of metabolite retention process of the system. A greater emphasis on the use of combination of micro array and proteomics techniques is needed to define the molecular targets for various micronutrients. Various techniques such as the serial expression of gene expression, protein arrays, and the evaluation of the mechanism will not only enhance our understanding of antiviral action at molecular level, but also help in finding the most effective strategy. Rational synthesis of the diverse derivatives with a more favorable profile activity can be of immense value along with the development of agent-selective endpoint markers. There is immediate need for crafting and executing an aggressive strategy involving nongovernmental organizations, chemists, microbiologists, clinicians, and experts with indigenous knowledge, failing which there are high chances of losing several untapped resources due to the extinction of plants. Combination studies/synergism is another area that has remained neglected. Further detailed studies to specify the minimum quantity of the phytochemicals to be consumed since the dosage of pure compounds effective in animals may not stay realistic when extrapolated to human system. By covering all the above gaps, it would be possible to strike a balance between the toxicity and the activity of a particular agent, which is essential for developing a new drug.

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