Antiviral Activity of Phytochemicals: A Comprehensive Review

Rajesh Naithani[1,*], Loredana C. Huma[1], Louis E. Holland[2], Deepak Shukla[3a,3b], David L. McCormick[1,5], Rajendra G. Mehta[1,5] and Robert M. Moriarty[1,4]

[1]*Drug Discovery Division, IIT Research Institute, Chicago, IL, USA;* [2]*Microbiology and Molecular Biology Division, IIT Research Institute, Chicago, IL, USA;* [3a]*Department of Microbiology and Immunology,* [3b]*Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, USA;* [4]*Department of Chemistry, University of Illinois at Chicago,* [5]*Carcinogenesis Chemoprevention Division, IIT Research Institute Chicago, IL, USA*

Abstract: Numerous numbers of biologically active agents have been identified for their diverse therapeutic functions. Detailed investigations of phytochemicals for antiviral activities have assumed greater importance in the last few decades. A wide variety of active phytochemicals, including the flavonoids, terpenoids, organosulfur compounds, limonoids, lignans, sulphides, polyphenolics, coumarins, saponins, chlorophyllins, furyl compounds, alkaloids, polyines, thiophenes, proteins and peptides have been found to have therapeutic applications against different genetically and functionally diverse viruses. The antiviral mechanism of these agents may be explained on basis of their antioxidant activities, scavenging capacities, inhibiting DNA, RNA synthesis, inhibition of the viral entry, or inhibiting the viral reproduction etc. Large number candidate substances such as phytochemicals and their synthetic derivatives have been identified by a combination of in vitro and in vivo studies in different biological assays. In this article we have made attempts to extensively review and provide comprehensive description of different phyto-antiviral agents. We have examined the recent developments in the field of plant derived antiviral agents. The major advances in the field of viral interactions in various biological assays have been summarized. In addition sources of origin, major viral studies mechanistic action and phase trials of various phytoantiviral agents have been included in the review.

Key Words: Flavonoids, antiviral, phytochemicals, virus, replication, inhibition.

INTRODUCTION

Throughout the human history man has been dependent on plant sources for their very basic needs. Use of medicinally active plants predates modern history. Over the course of human evolution thousands of biologically active plant products have been identified and they form an integral part of the traditional medicinal systems, all around the world. The general interest of consumer in natural products is evident by the multibillion dollar market of health supplements. It is widely accepted that phytochemicals are nontoxic when used in smaller amounts and manifest a wide range of biological activities.

A World Health Organization report has indicated the dependence of over 80% of world's population on traditional plants to meet their health requirement [1]. Plants have the ability to synthesize a wide array of compounds. Polyphenolics or flavonoids constitute the largest group of substances that are widely present in plant kingdom; more than 8000 are known to date [2]. As has been shown the chemical nature of flavonoids variation in the structure occurs around the heterocyclic moiety. The vast structural diversity of polyphenols makes them ideally suited for antiviral research. Flavonoids have a unique template such as chalcone, flavonone, flavone associated with a large number of biological activities. Radical quenching ability of the phenolic groups makes it highly interesting to be used in drugs and food preservative [3]. Overall evidence available in the literature reveals that numerous flavonoids have excellent antiviral activity [4, 5]. Beside flavonoids, there are several other secondary metabolites in plants. Under stress plants produce an important category of compounds called phytoalexins which are also considered to be antibiotics produced by plants [6]. Phytoalexins tend to fall into several classes including terpenoids, glycosteroids and alkaloids. In addition, plants have various other constituents like organosulfur compounds, vitamins, polyunsaturated fatty acids, quinones, lactoferrin, lignans, tannins which have been studied for antiviral activity in detail. These products may be responsible for providing protection (phytoalexins, lignan) characteristics odor (terpenoids), color (quinones), and flavor (curcumin, organosulfur compounds) to the plants.

For centuries preparations derived from plants have been used against number of diseases. Over the last few decades natural products have been studied for anti-infective and more specifically, anti-viral activities. Basic researches in experimental models using various biological systems strongly suggest the protective role of plant derived natural compounds against viral infection [6-10]. Although several hundred plants species have been evaluated as novel antiviral agents, additional work is still needed since viral infections are fast becoming a bigger threat to humankind. In this regard, medicinal plants are increasingly being projected as suitable alternative sources of antiviral agents. Unfortunately many antiviral compounds presently in clinical use have a narrow spectrum of activity, limited therapeutic usefulness and variable toxicity. Whether natural antivirals will emerge

*Address correspondence to this author at the Department of Chemistry, University of Illinois at Chicago, Chicago-60607, USA;
E-mail: rajesh. naithani@gmail.com

1389-5575/08 $55.00+.00

as a viable alternative medicine or a synergistic combination therapy with pre-existing antiviral therapy will depend on identifying broad-spectrum plant-based antiviral combined with a method of delivery that ensures its stability and bioavailability. In addition, the development of a suitable *in vitro* pharmacodynamic screening technique could contribute to rapid identification of potential bioactive plants and also to the standardization and/or pharmacokinetic-pharmacodynamic profiling of the bioactive components.

There is also an emerging problem of development of resistant viral strains [11, 12]. Most of the well known drugs approved for viral infection derive origin from nucleosides or closely related carbon chain with amine [13]. Emergence of many new and perhaps more deadly viruses such as Ebola and Marburg viruses and possible threat of their use as arsenal for bioterrorism have enhanced our urgency to find new and potent anti-virals 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]. It is well known fact that in the past efforts have been directed to alter the structure of parent compound in order to counter the resistivity of virus. However these modifications cannot be endless. It's imperative that new classes of drug should be developed having different target sites.

The antiviral mechanism of these agents may be by antioxidant activities, scavenging capacities, inhibiting DNA, RNA synthesis, inhibition of the viral entry, or inhibiting the viral reproduction etc. Various gaps still exist in our understanding the mechanism of natural antivirals. There are a large number of plants derived molecules which exhibit antiviral activity but their mechanistic action needs to be explored.

Apart from screening of the naturally occurring compounds several groups have successfully designed and synthesized novel analogs having promising antiviral activity. The main aim of the structural modification is to reduce toxicity, increase bioavailability in comparison to the parent molecule. Viruses absolutely require host cell environment for survival. The invasion strategy may be different for each virus. Since medicinal plants have an endless variety of chemical constituents, it could be utilized to inhibit the replication of both DNA and RNA virus.

In this review we examine the current literature on various naturally occurring compounds with antiviral activity. Besides collection of literature from various independent sources, searches were performed on Scifinder, Pubmed etc. The major advances in the field of growth inhibition of specific viruses in different biological assays have been summarized. In addition sources of origin, mechanistic action and phase trials of various plants derived antiviral agents have been included in the review.

MAJOR GROUP OF ANTIVIRAL COMPOUNDS FROM PLANT

1. Flavonoids

Flavonoids are easily recognized as flower pigments in most angiosperm families (flowering plants). However, their occurrence is not restricted to flowers. The flavonoid structure is basically a polyphenol consisting of 15-carbon atoms skeleton (**1**). The further classification of flavonoids is based upon the oxidation and the substitution pattern of the ring C. The skeleton of the flavonoid can be represented as the C_6-C_3-C_6 system. The structure **1** (Fig. **1**) illustrates the numbering of the flavonoid structure. In a few cases, the six-membered heterocyclic ring C is replaced by a five-membered ring. The C_2 of the carbon atom is directly linked to the oxygen as a result of which furan type molecule is formed called aurone (**2**). The class flavonoids constitute the largest source of antiviral agents in the entire plant kingdom. Flavonoids have also been known to posses biochemical effects, which inhibit a number of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca **+2** -ATPase, lipoxygenase, cycloxygenase, etc. They also have a regulatory role on different hormones like estrogens, androgens and thyroid hormone. Thomas *et al.* evaluated flavonoids for activity against herpes simplex virus andreported that flavonols are more active than flavones and the order of importance was galangin > kaempferol > quercetin [15a**]** . Natural plant flavonoid polymer (of molecular weight 2100 Daltons) was found to have antiviral activity against two strains of type-1 HSV and type -2 herpes simplex virus [15b]. Emphasizing the role of hydroxyl group attached to the flavone moiety Gerdin *et al.* found that out of twenty-eight fiavonoids, flavan-3-o1 was more effective in selective inhibition of HIV-1, HIV-2 and similar immunodeficiency virus infections [15c].

CLASSIFICATION OF FLAVONOIDS

1.1. Chalcones

Chalcones is one of the major subclass of flavonoids, which have been extensively studied for antiviral activity. Chalcones are aromatic ketones i.e. 1,3-Diphenylpropenone (benzylideneacetophenone) and its derivatives formed by substitution. The general formula ArCH=CHC(=O)Ar forms the central core for a variety of important biological compounds, which are collectively known as chalcones. Chalcones, considered as the precursors of flavonoids and isoflavonoids, are abundant in edible plants, and have also been shown to display a diverse array of pharmacological activities. Deng *et al.* have reported excellent antiviral activity of chalcones **3** and **4** [15d]. In order to further utilize the chalcones,the authors developed pharmacophore models to identify chemical signatures considered important for the antiviral activity. The vaildation of the derived models was achieved with a collection of published inhibitors, and then were applied to screen a subset of our small molecule database. Forty-four compounds showed inhibitory potency<100 microM, and four of them exhibited IC50 values<10 microM. An excellent overview of the pharmacological activity of synthetic and naturally occurring chalcones appeared recently. A complementary to earlier reviews and this review deals with the more recent reports and structure activity relationship on antiviral chalcones [15e].

1.2. Dihydrochalcones

Dihydrochalcones (**5**) are a group of compounds derived from chalcones by the reduction of the double bond. This reduction destroys the ability of chalcone chromophore as far

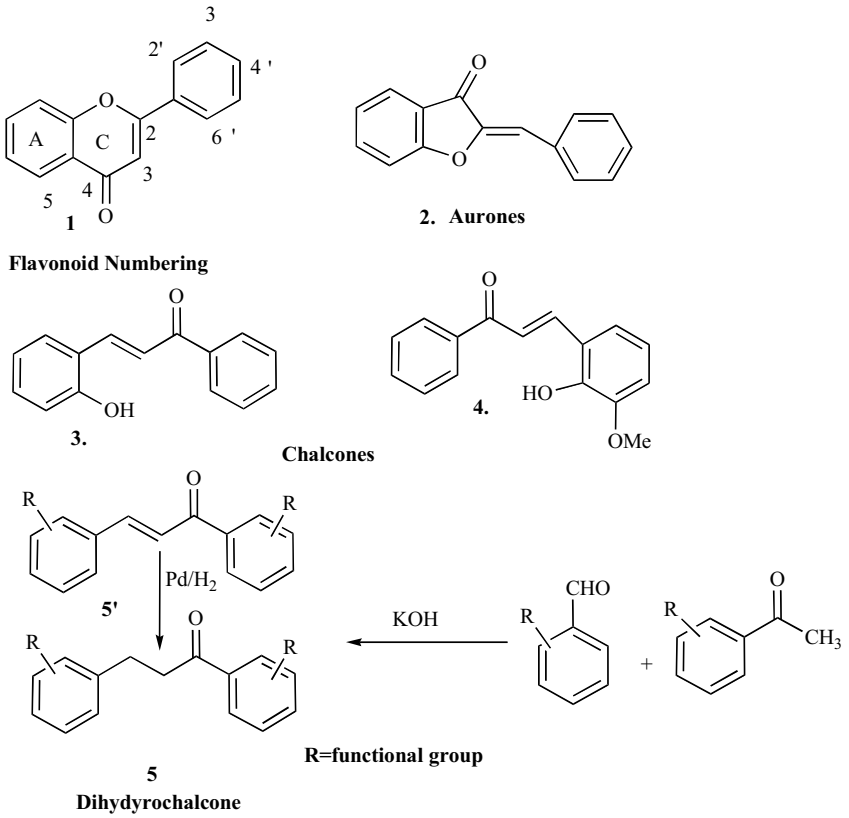


Fig. (1).

as the UV visibility is concerned; even it's not apparent as are their parent molecules, particularly on paper or thin layer chromatograms. General method of synthesis of dihydrochalcones (**5**) is outlined in Fig. **1**. Dihydrochalcones derived from *Millettia leucantha KURZ (Leguminosae)* showed anti-herpes simplex virus (HSV) activity [16].

1.3. Flavones

Flavones constitute a major class in flavonoid family having the characteristic 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one) backbone and are characteristically found in *Lamiaceae*, *Apiaceae, Asteraceae* 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**) [17]. Prendergast *et al.* have used 3',4'-diacetoxy-5,6,7-trimethoxyflavone or naringin (**7**) in the treatment of infections, particularly for viral (e.g., HCV, HIV, a picornavirus genus virus or a respiratory virus) or parasite (e.g., toxoplasmosis) infections [18]. In a structure activity study, Mishra *et al.* utilized molecular electrostatic potential (MEP) maps to study the anti-picornavirus activities of methoxy flavones. Geometries of the molecules were optimised and charge distributions computed using the AM1 molecular orbital method. The authors proposed that anti-picornavirus activities of the flavones is 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 C7 atom of the molecules [18b].

1.4. Flavonone

Characterized by the carbonyl group at the C_4 carbon the double bond of the flavones is reduced. Moriarty *et al.* synthesized several novel analogs of flavanone Abyssinone II (**8**), a naturally occurring prenylated flavanone, and tested for antiviral activity in Hela 5 cells using a recombinant a-galactosidase expressing strain of HSV-1(Herpes simplex virus Type 1) and reported substantial antiviral activity of the analogs [19].

1.5. Dihydroflavonols

Dihydroflavonols (**9**) are characterized by hydroxyl group at position C_3 of the flavonone molecule. The flavanonol compounds or their mixture are applied for treating and preventing hepatitis B, mycotic infection, liver protection, inflammation disease, autoimmune disease [20].

1.6. Flavonol

Flavonol is characterized by the presence of OH group on position 3 of the flavone. 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, parainfluenza virus type 3 (Pf-3), and respiratory syncytial virus (RSV) was studied *in vitro* 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 medium

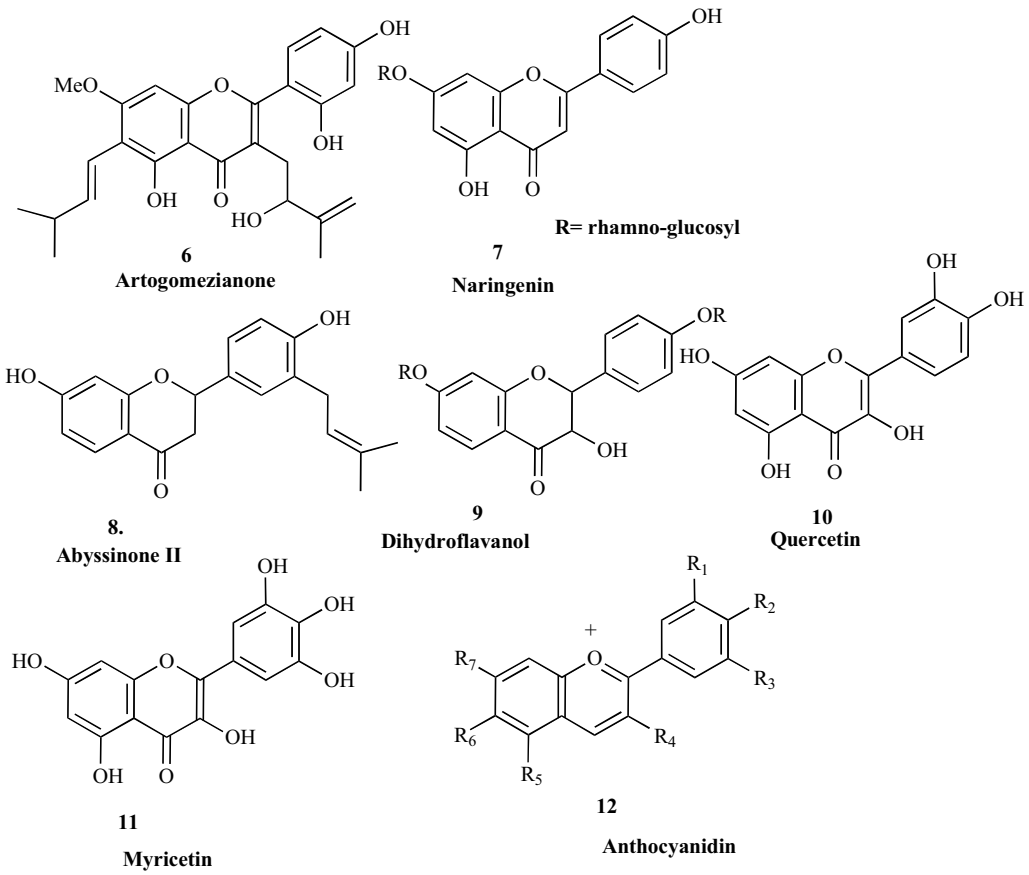


Fig. (2).

containing quercetin [21]. 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 [22].

1.7. Anthocyanidin

Anthocyanidin (**12**), an important group of plant pigments is constituted of the aglycone (anthocyanidine) and the glycone (sugar part) The fact that free OH group can coordinate with metal ions like Ca^{2+} and Mg^{2+} under alkali conditions is responsible for various biological activity displayed by these kind of molecules. Anderson *et al.* have reported the therapeutic effect of anthocyanidin treatment of cancer, diseases caused by lesions in connective tissues, and diseases caused by viruses [23]. Various substituent in different anthocyanidins have been tabulated in Table **1**.

Isoflavonoids

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**.

1.8. Isoflavones

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. [24]

1.9. Isoflavanones

Isoflavanones bear the same relationship to isoflavones as flavanones do to flavones. And, as in the case of flava-

Table 1.

Anthocyanidin (12)	R1	R2	R3	R4	R5	R6	R7
• Aurantinidin	H	OH	H	OH	OH	OH	OH
• Cyanidin	OH	OH	H	OH	OH	H	OH
• Delphinidin	OH	OH	OH	OH	OH	H	OH
• Europinidin	OCH_3	OH	OH	OH	OCH_3	H	OH

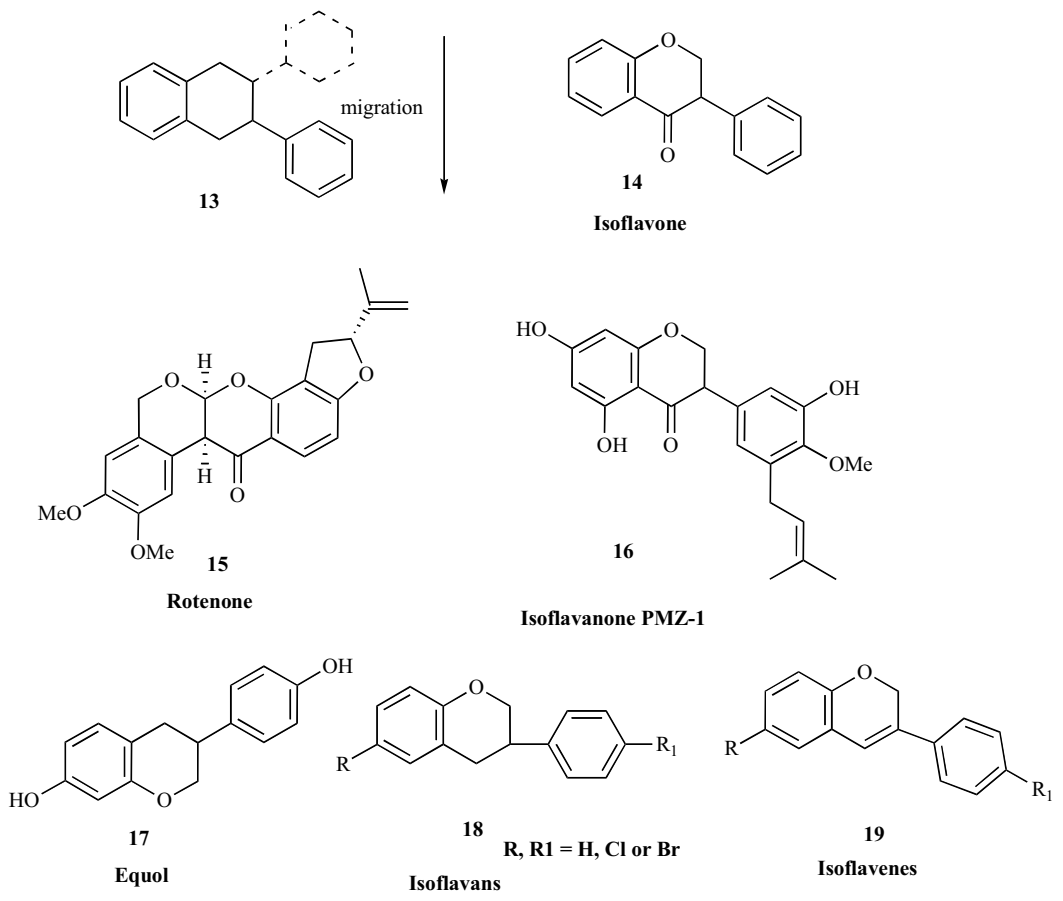


Fig. (3).

nones, isoflavanones have a chiral center (C_3 in isoflavanones). PMZ-1, a prenylated isoflavonone (**16**), isolated *Bolusanthus speciosus* (Bolus Harms) has exhibhited excellent activity against HIV-having a broad therapeutic index (TI > 300) [25].

1.10. Isoflavans and Isoflavenes

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, e.g. 7,4'-dihydroxyisoflavan (17, equol). The effect of substituted isoflavans (**18**) (R and R1 = H, Cl, or Br) and isoflavenes (**19**) on HRV 1B infection of HeLa cells was examined by Conti and coworkers who found that the drugs inhibited virus plaque formation in cell cultures with isoflavans being more effective than isoflavenes [26]. It was found that the cells pretreated with compounds before challenge with HRV -1 b exhibited resistance to the virus induced cytopathic effect. It was further shown that the antiviral state induced by the most active compounds persisted for at least 10 h and did not appear to be mediated by interferon production.

1.11. Arylcoumarins

The arylcoumarins are characterized by the presence of a carbonyl function at C_2 and they may or may not have oxygenation at C_4. Large number of coumarins has been studied for the antiviral activities [27]. 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 [28].

1.12. Flavans

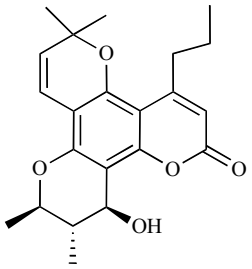
Compounds belonging to class flavans are normally devoid of carbonyl group at position 2. Although this class of compounds contains some common and comparatively simple compounds, catechin and epicatechin in particular, the overall structural complexity of the group is impressive. Two new antiviral flavan 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**) [29].

1.13. Neoflavonoids

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. Structure of the 4 neoflavonoids. A series of inophyllums **22** – **25** were isolated from the Malaysian tree *Calophyllum inophyllum* and evaluated for inhibitory activity against HIV-1 RT. Among them, the most active compounds, inophyllum B and inophyllum P showed IC_{50} values against RT of 0.038 and 0.130 mM, respectively [30].

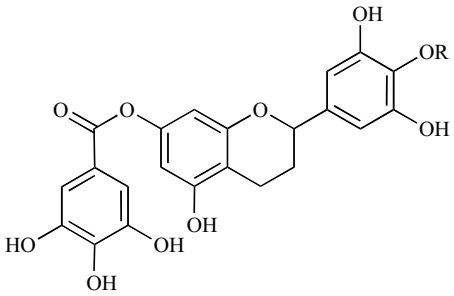
2. Alkaloids

Alkaloids are compounds containing nitrogen in a heterocyclic ring that are common to about 15 to 20% of all



20

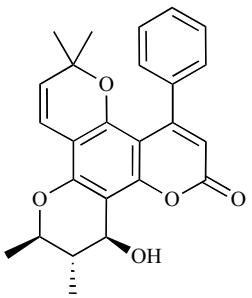
calanolide A



21

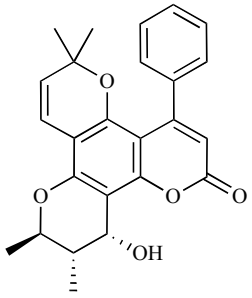
(a) 7-Ogalloyltriacetiflavin (R=H)

(b) 7,4' O-di galloyltriacetiflavan (R=$COOC_6H_5$)



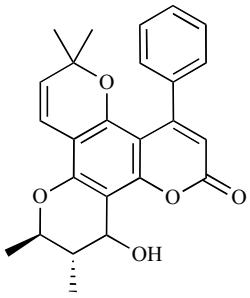
22

(+) inophyllum B



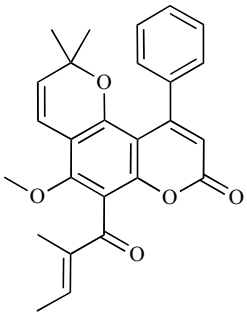
23

(+) inophyllum P



24

(+) inophyllum C



25

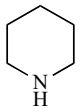
calophyllolide

Fig. (4).

vascular plants. They are synthesized by plants from amino acids. 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 [31]. In an attempt to relate the structure with the antiviral activity Houghton *et al.* tested several naturally occuring chromone alkaloids (derived from rootbark of *Schumanniophyton magnificum*) for 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 favour the anti-HIV activity. Irreversible binding to gp 120 was considered to be responsible for the anti-HIV activity [31 b].

3. Terpenoid

The terpenoids, sometimes referred to as isoprenoids, are a large and diverse class of naturally occurring organic chemicals derived from five-carbon isoprene units, which are assembled and modified in thousands of ways. Terpenoids can also be classified according to the number of cyclic structures they contain. In this study, numerous phytocompounds were evaluated for activity against anti-severe acute respiratory syndrome associated coronavirus (SARS-CoV) activities using a cell-based assay measuring SARS-CoV-induced cytopathogenic effect on Vero E6 cells and compounds (**30-32**) showed excellent activities [32]. This recent study, included 221 phytocompounds (including ten diterpenoids, two sesquiterpenoids, two triterpenoids) were evaluated for activity against anti-severe acute respiratory syndrome associated coronavirus (SARS-CoV) activities utilizing a cell-based assay measuring SARS-CoV-induced cyto-

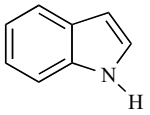


26

Piperidine

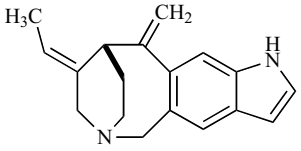
27

Pyridine



28

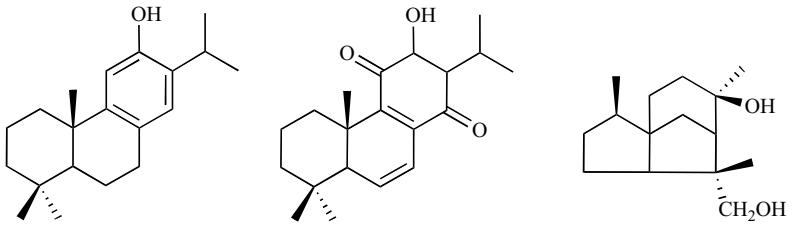
Indole



29

Pericalline

Fig. (5).



30 **31** **32**

Ferruginol **6-7-dehydroyleanone** **3-beta cedran**

Fig. (6).

pathogenic 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, lupane-type triterpenes.

4. Carotenoids

Carotenoids 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 and act as backbone to molecule. Carotenoids can be Xanthophylls (molecules containing oxygen) such as lutein and zeaxanthin and carotenes (the unoxygenated or oxygen free carotenoids) concentrations of plasma carotenoids are associated with increased risk of death during HIV infection among infants in Uganda carotenoids (α-carotene (**33**), β-carotene (**34**), lutein/zeaxanthin (**35**) and lycopene (**36**) [33].

5. Organosulfur Compounds

Sulfur containing compounds are present in all *Brasicaceae* family vegetables and plants belonging to *Allium* family (Table **2**) constitute an important class of antiviral agents [34].

There are number of representative examples of organosulfurs antivirals (**37-40**). The only drawback organosulfur compounds have is a pungent odor, and chemical instability. Several unsymmetrical aralkyl disulfides,were synthesized and oxidized to study the relatively unexplored class of thiolsulfinate[34b].

6. Vitamins

It has been shown that vitamin C (**41**) can increase the host immune response, and this may provide protection against infectious diseases [35]. Vitamin E supplementation might be effective in the treatment of chronic hepatitis B [35b]. The name vitamin E covers a collection of eight fat soluble compounds, tocopherols (**42**) (methyl derivatives of tocopherol) and tocotrienols (**43**).

7. Selenium Compounds

Few studies have indicated the importance of selenium compounds (**44-46**) as antiviral agents. The data generated from experimentation on various animal model and cell lines demonstrates significant beneficial effect of selenium on different viral infections. Cermelli *et al.* studied the antiviral effects of three selenium compounds on replication of Coxsackie virus B_5 replication [36]. 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 analogues of ebselen for their activity in *in vitro* antiviral assay . Some of the analogs tested had an appreciable inhibition of cythopatic activity of herpes simplex virus type 1—HSV-1 and encephalomyocarditis virus—EMCV [36b].

MISCELLANEOUS

8. Curcumin and its Derivative

Turmeric is one of the key constituent of food in the Indian subcontinent. Curcumin (**47**) derived from turmeric. Curcumin-like derivatives have shown potent activity against HIV-1 integrase [37].

9. Chlorophyllin

Chlorophyllin (CHLN) (**48**, **49**) is a synthetic derivative of chlorophyll that possesses antimutagenic activity against several environmental contaminants. In the present study, CHLN was assayed for its capacity to prevent nuclear fragmentation (NF) in HEp-2 cells infected with poliovirus [38].

10. Chitin and Chiotosan

Chitin is one of the most abundant polysaccharide found in nature. Chitin the polysaccharides polymer consists of the aminosugar N-acetylglucosamine which is partially deacetylated. The mostly deacetylated form of chitin is called chitosan. Carboxymethyl chitin with a 7.66% degree of sulfation (SCM-chitin III) showed a significant inhibition of Friend murine leukemia helper virus (F-MuLV) and HSV, However there was no effect on Sendai virus growth [39].

11. Tannins

The word tannin is very old and reflects a traditional technology. "Tanning" (waterproofing and preserving) was the word used to describe the process of transforming animal hides into leather by using plant extracts from different plant parts of different plant species. Tannin is basically a phenolic compound of sufficiently high molecular weight containing sufficient hydroxyls and other suitable groups (**50**) (i.e. carboxyls) to form effectively strong complexes [40]. Seven ellagitannins isolated from *Phyllanthus myrtifolius* and *P. urinaria (Euphorbiaceae)* have been shown to be active against Epstein-Barr virus DNA polymerase (EBV-DP).

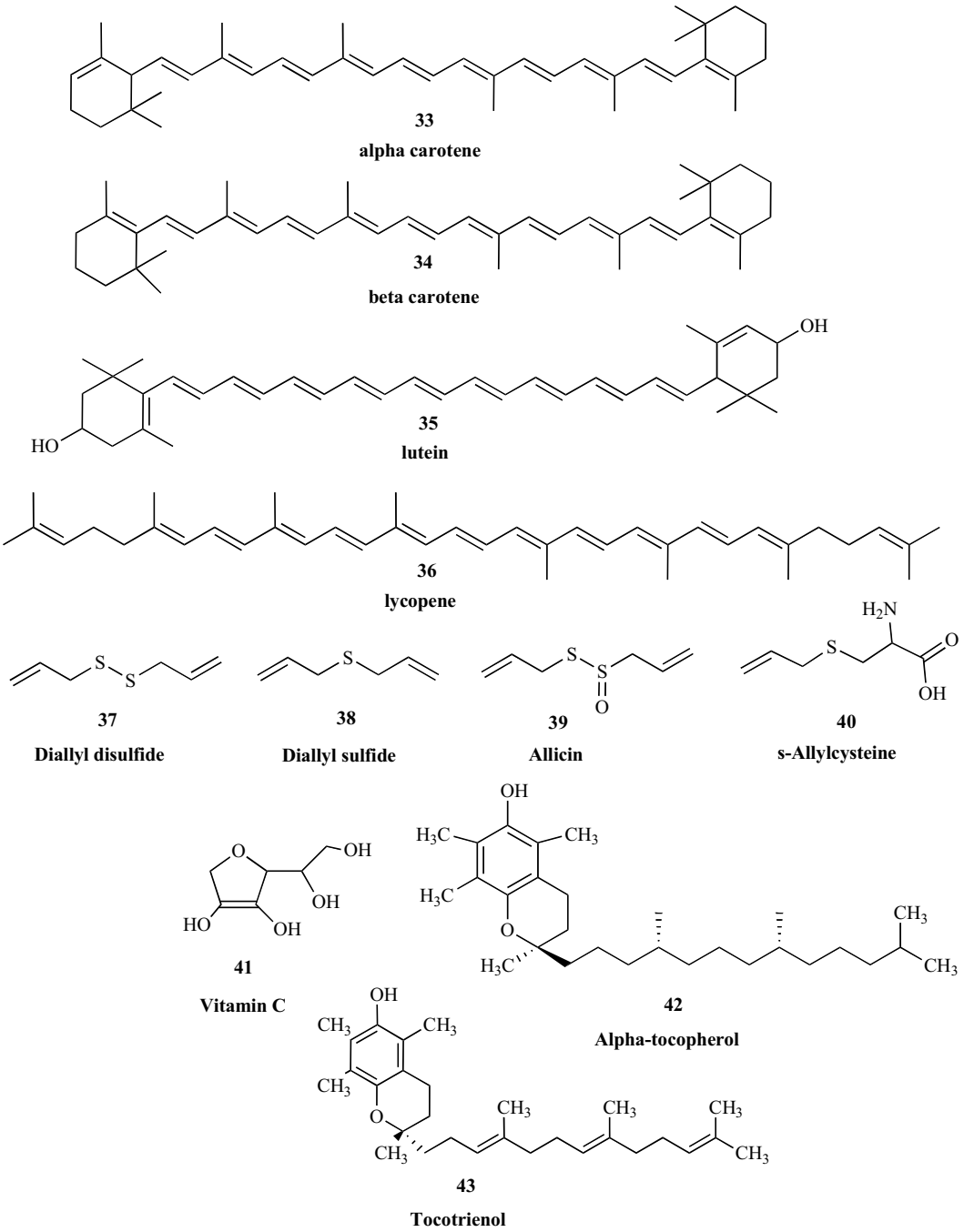


Fig. (7).

Table 2.

S/No	Type of Compounds	Compounds	Natural Source
1	Sulfides	Dithiolethiones Allyl sulfides	Broccoli, Garlic, Onion
2	Isothiocyanates	Sulphoraphanes Phenylethyl isothiocyanates	Cauliflower, Cabbage, Kale, Bok choy, Brussels sprouts, Radish Mustard, water garden cress
3	Glucosinolates	Glucobrassinin	Cauliflower, Cabbage, Kale, Bok choy, Brussels sprouts, Radish Mustard, Water garden cress

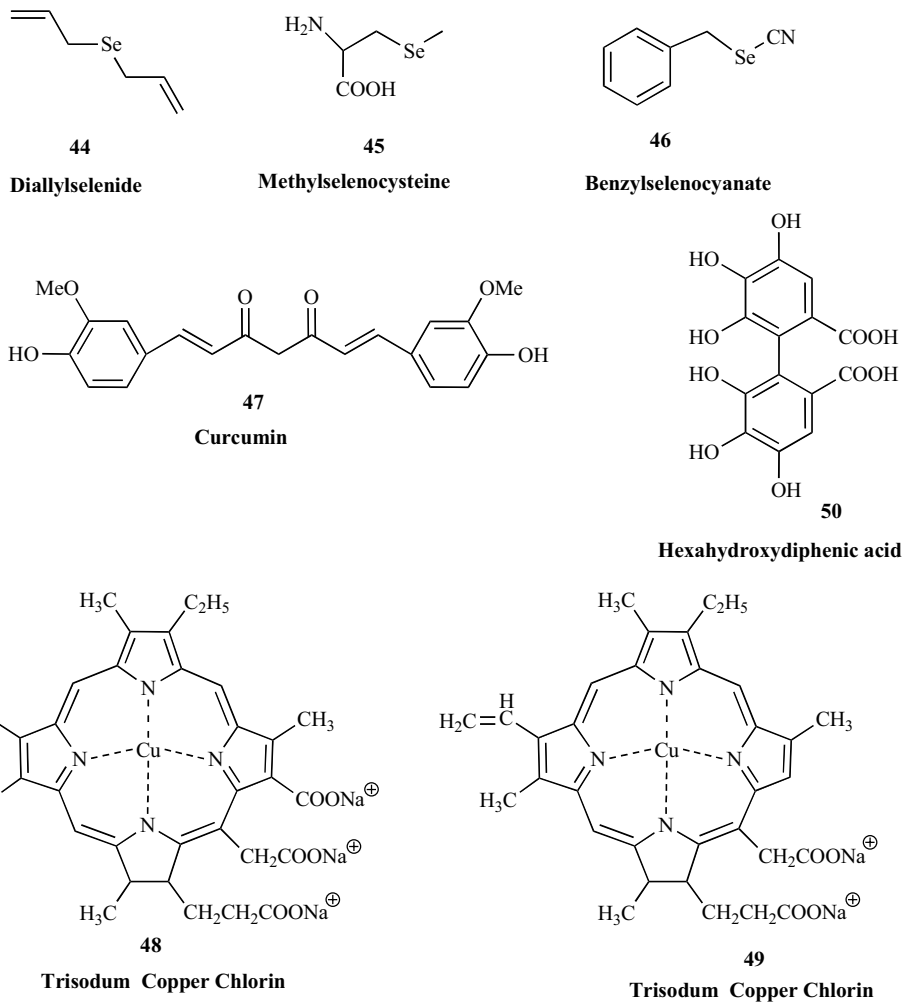


Fig. (8).

12. Polyacetylenes

The polyynes are a group of organic compounds with alternating single and triple bonds. The molecules have characteristic two or three conjugated alkynyl groups, with various terminal functional groups for solubility and biological activity (**51**, **52**). Polyacetylenes fall in the hydrocarbon category that strongly absorb long-wave UV light; their medicinal activity 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 [41] that is augmented by UV light exposure. The polyacetylenes are one of the few natural substances reported to inhibit CMV, a type of herpes virus that causes disease in immune-compromised individuals. Importantly, these polyacetylenes to not cause DNA changes (as do other herbal photoactivated substances, such as furanocoumarines 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 [42].

13. Polysaccharides

Polysaccharides are relatively complex carbohydrates having a general formula of $C_n(H_2O)_n$ (**53**) where n is usually a large number between 200 and 2500. The general formula can also be represented as $(C_6H_{12}O_6)_n$ where n=40-3000. A number of highly sulfated red algal polysaccharides have been found to possess antiviral activity against a variety of animal viruses [43]. The potential antiviral activity of algal polysaccharides was first shown by Gerber *and* colleagues, who observed that the polysaccharides extracted from *Gelidium cartilagenium* afforded protection for embryonated eggs against influenza B and mumps viruses [44]. Huleihel *et al.* evaluated antiviral effect of polysaccharides extracted from several species of red microalgae. These polysaccharides are highly sulfated and consist mainly of xylose, glucose and galactose. The cell-wall sulphated polysaccharide of the red microalga *Porphyridium* sp. has impressive antiviral activity against *Herpessimplex* viruses types 1 and 2 (HSV 1, 2) and *Varicella zoster virus* (VZV) [45, 46].

14. Lignan

Lignans are one of the major classes of phytoestrogens, which are estrogen-like chemicals and also act as antioxidants. [47]: Nordihydroguaiaretic acid (NDGA) (**54**) is a lignan present in the perspired resin of leaves of *Larrea divaricata*. This compound and its derivatives have *in vitro* inhibition against several viruses including HIV, herpes simplex I and II, and human papilloma [48]. The pharma-

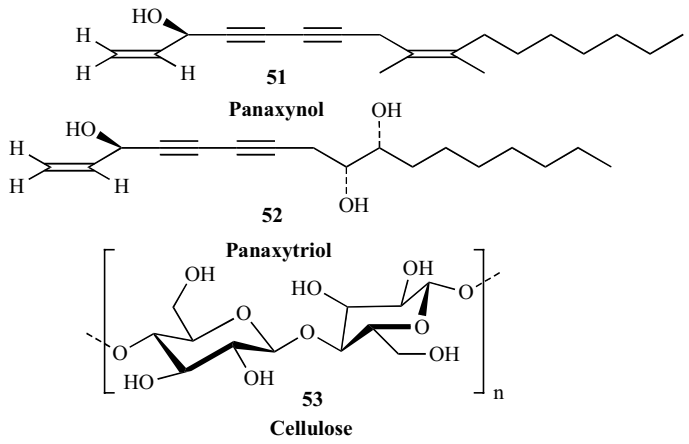


Fig. (9).

cokinetics and metabolism of retrojusticidin B, an anti-HIV reverse transcriptase agent isolated from *Phyllanthus myritifolius,* were studied in rats.

15. Anthraquinone

Anthraquinone (**55**) (9, 10-dioxoanthracene) is an aromatic organic compound derivative of anthracene. Chrysophanic acid (**56**) (1,8-dihydroxy-3-methylanthraquinone), isolated from the Australian aboriginal medicinal plant *Dianella longifolia,* has been found to inhibit the replication of poliovirus types 2 and 3 (*in vitro*). Severe acute respiratory syndrome (SARS) is an emerging infectious disease which has attracted global attention is caused by a novel coronavirus (SARS-CoV). 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). Emodin (**57**), an anthraquinone compound 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 [49, 50].

16. Gingerols

Gingerols (**58**) have (derived from ginger, a typical south asian spice) traditionally been used to cure common colds and throat infections and form an important constituent of Ayurvedic formulations. There have been numerous studiers on the efficacy of these compounds as antiviral agents [51].

17. Salicylic acid

Salicylic acid (**59**) is the chemical compound with the formula $C_6H_4(OH)CO_2H$, where the OH group is adjacent to the carboxyl group. This colorless crystalline organic acid is widely used in organic synthesis and functions as a plant hormone. It has been shown that salicylic acid (SA) can induce resistance to a wide range of pathogen types. In the case of viruses, SA 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 [52].

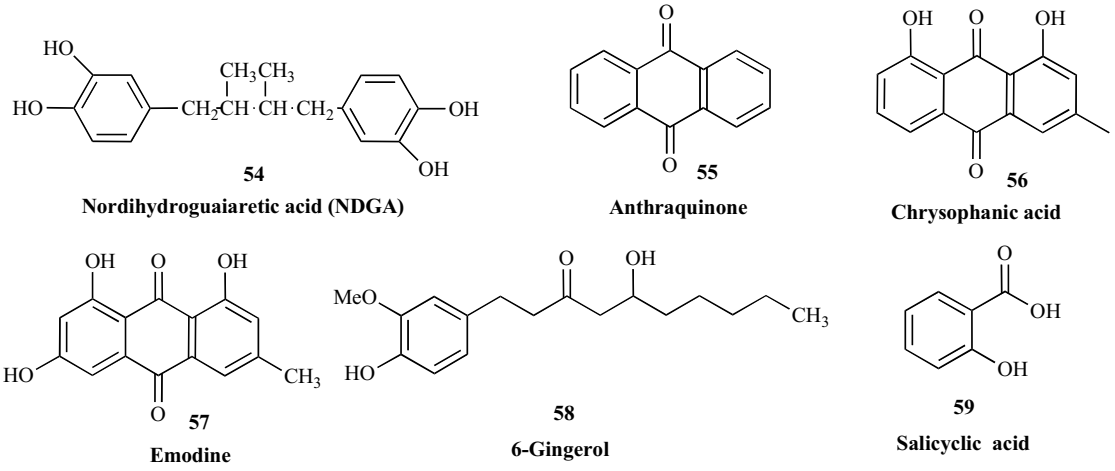


Fig. (10).

ANTIVIRAL MECHANISTIC ASPECTS OF PHYTOCHEMICALS

The antiviral activities of phytochemicals have been recognized for more than four decades. Studies have indicated that antiviral action of plant derived products may be attributed to number mechanisms (Table **3**). Application of nature derived agents to human is advantageous since most of these have all most no side-effect. The action of these agents may be explained on the basis of following well accepted mechanisms. It is possible that antiviral effect of the compound may be explained on basis of more than one mechanism. In addition there are compounds exhibhiting promising antiviral activities but their action of mechanism has not been elucidated so far.

SUMMARY OF THE MECHANISM OF VARIOUS PHYTOCHEMICALS AS ANTIVIRAL AGENTS

Viral Studies

Investigations of phytochemicals for antiviral activities have assumed greater importance in recent times. There have been numerous *in vitro* studies supporting antiviral activity of phytochemicals. In order to 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. There is tremendous amount of literature available regarding antiviral potential of phytochemicals. Owing to the space constraint, we have limited our discussion to major antiviral evaluations utilizing phytochemicals. For the sake of clarity, the discussion has been classified into different sections with a focus on viral diseases.

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. As of year 2006, AIDS has killed more than 25 million people, since it was first recognized in 1981 [84a]. With the recent advances in understanding the biology of HIV there has been increased focus on usage of phytochemicals as antivirals against HIV. In continuation of search for novel and effective HIV protease inhibitors, several diterpenes were evaluated and it was reported that carnosic acid (**60**) displayed the strongest inhibitory effect (IC90 = 0.08 micrograms/ml). The same compound was also assayed against HIV-1 virus replication (IC90 = 0.32 micrograms/ml). [84b]. Isoobtusitin (**61**), a prenylated coumarin, having good activity against polio virus was also tested for its *in vitro* inhibitory activity against the replication of the HIV virus, however in this case inhibition was only 10% . [85]. Tannins from different plant sources showed potent inhibitory activity on HIV-1 replication, HIV-1-mediated cell fusion, and the gp41 six-helix bundle formation. It was also shown that tannin inhibited HIV-1 entry into target cells by interfering with the gp41 six-helix bundle formation, thus blocking HIV-1 fusion with the target cell [86]. Anti- HIV activity of mulberry juice was studied in detail by Sakagami and colleagues who found excellent anti-stress and anti-HIV activity in different fractions of the juice. [87]. A novel anti-HIV alkaloid drymaritin (**62**) isolated from *Drymaria diandria* has shown anti-HIV effect in H9 lymphocytes with EC50 value of 0.699 μg /ml [88]. Lee *et al.* have shown that flavonoid glucuronide isolated from flowers of *Chrysanthemum morifolium* 7-O-beta-D-(4"-caffeoyl)glucuronide derived from apigenin (**63**) displayed strong HIV-1 integrase inhibitory activity (IC 50 = 7.2±3.4 μg/mL) and anti-HIV activity in a cell culture assay (EC 50 = 41.86±1.43 μg/mL) using HIV-1IIIB infected MT-4 cells [89].

Olivero and co-workers evaluated a number of polyphenols such as ferulic acid, gallic, caffeic, furulate, gallate, curcumin and have reported upto 80% inhibition of HIV replication [90]. Several flavonoids derived from *Plantago asiatica* were tested as potential HIV reverse transcriptase inhibitor. Scutellarein and 6-hydroxyluteolin displayed strong HIV reverse transcriptase inhibition, along with glycosides plantaginin and 6-hydroxyluteolin 7-glucoside. [91]. Kim *et al.* reported excellent inhibitory activities of flavonol glycoside gallate ester against human immunodeficiency virus. Bioassay-directed chromatographic fractionation of an ethyl acetate extract of the leaves of *Acer okamotoanum* using HIV-1 integrase afforded a new acylated flavonol glycoside, quercetin 3-O-(2",6"-O-digalloyl)-beta-D-galactopyranoside, together with six known flavonol glycosides and three known phenolic compounds.. The most active compounds were quercetin 3-O-(2"-galloyl)-alpha-L-arabinopyranoside and quercetin 3-O-(2",6"-O-digalloyl)-beta-D-galactopyranoside, which exhibited IC50 values of 18.1 +/- 1.3 and 24.2 +/- 6.6 micrograms/mL, respectively, against HIV-1 integrase [92].

The role of chrysin, apigenin (**63**) and acacetin (**64**) in models of latent infection was evaluated and it was suggested that these flavonoids inhibit HIV-1 activation. The three compounds had favorable potencies against HIV activation in relation to their growth inhibitory effects (therapeutic index 5-10) [93]. Mahmood and colleagues examined 28 flavonoids for virus infectivity inactivation. It was found that the flavans were generally more effective than flavones and flavanones in selective inhibition of HIV-1, HIV-2 or simian immunodeficiency virus infection. Mechanistic studies have revealed the binding of sCD4 and antibody to gp120. This indicates that the effective compounds interact irreversibly with gp120 to inactivate virus infectivity and block infection [94]. As a part of the study to evaluate the efficiency of the anthraquinones, derived from *Hypericum* different anthroquinones with substituents such as hydroxyl, amino, halogen, carboxylic acid, substituted aromatic group, and sulfonate were tested for their activity against human immunodeficiency virus type 1 (HIV-1) in primary human lymphocytes. Among all the compounds that were screened, it was noticed that the polyphenolic and/or polysulfonate substituted anthraquinones possessed most potent antiviral activity. An anthraquinone was also found to inhibit HIV-1 reverse transcriptase. However, this enzyme inhibition was selective only for 1,2,5,8-tetrahydroanthraquinone and hypericin. Due to its substantial anti-viral property, this species have a potential for the treatment of Acquired Immuno Deficiency Syndrome (AIDS) as a hepatoprotectant [95].

Table 3. Antiviral Properties of Phytochemicals

	Name of the Phytochemicals /Class	Details of Study	Virus	Mechanism	References
1	Flavone (4', 5-dihydroxy 3,3',7-trimethoxy flavone)	Effect of flavone on replication	Human: • Picornaviruses • Rhinoviruses • Coxsackieviruses	Replication inhibition, selective inhibition of viral RNA synthesis in the cell culture	Ishitsuka *et al.*, [53]

2	Polyphenolic complex (PC) containing:
	• Catechins
	• Flavonoids
	  • Kaempferol
	  • Myricetin
	  • Monne
	  • Quercetin
	  • Ramnasin
	  • Retusin
		Effect of PC on the expression of viral proteins on the surface of virus infected cells. The expression of viral proteins haemagglutin (HA), neuraminidase (NA) and nucleoprotein (NP) on the surface of infected cells was investigated by ELISA with monoclonal antibodies		PC inhibited total viral protein synthesis and the synthesis of HA, NA and NP as shown in single cycle of virus replication experiments.	Serkedjieva *et al.* [54]

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 that flavonoids interfere with an early event in the virus life cycle resulting in decreased infectivity and titre in tissue culture.	Malhotra *et al.* [55]
4	BCA, BA	Elucidation of mechanism of the anti-viral effect of BA	HIV-1	BA inhibits HIV-1 infection at the level of viral entry. Similar inhibitory effect was observed with another flavonoid Baicalein (BCA) which is structurally related to BA	Li *et al.*, [56]
5	BCA, Genistein	Investigation of antiviral activity of baicalin and genistein against human cytomegalovirus	HCMV	Primary mechanism of action for baicalein may be to block 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.*, [57]

(Table 3. Contd….)

	Name of the Phytochemicals /Class	Details of Study	Virus	Mechanism	References
6	3-Methylquercetin	Effect on methylquercetin on poliovirus replication.	Poliovirus	Blocks viral replication, selective inhibition of poliovirus RNA synthesis both in infected cells and in cell-free systems.	Castrillo *et al.*, [58]

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 *et al.*, [59]
					• Sydiskis *et al.*, [60]
					• Kurokawa *et al.*, [61]
					• Liu *et al.*, [62]

8	• Quercetin (Q) • Luteolin (LU) • 3-O-methylquercetin (3MQ)	Effect on the viral replication cycle of HSV-1	HSV-1	Interferes with the events occurring between the third and ninth hour of HSV-1 replication cycle, which includes transcription and translation of viral proteins.	Bettega1 *et al.*, [63]
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.*, [64]
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.*, [65]
11	• (−)EGCG • (−) ECG • (−) EGC	Effect on viral synthesis	Influenza virus	Quantitative RT-PCR analysis revealed that, at high concentration, EGCG and ECG also suppressed viral RNA synthesis in MDCK cells whereas EGC failed to show similar effect only by specific interaction with HA, but altering the physical properties of viral membrane.	Song *et al.*, [66]

(Table 3. Contd….)

	Name of the Phytochemicals /Class	Details of Study	Virus	Mechanism	References
12	Flavonoids complex: • Amentoflavone • Theaflavin • Iridoids • Phenylpropanoid glycosides • Agathisflavone • Robustaflavone • Rhusflavanone • Succedaneflavanone • Chrysosplenol C • Morin • Coumarins • Galangin (3,5,7-trihydroxyflavone) • Baicalin • Quercetin • Isoquercetin	Effect on viral replication	HIV	Blockage of RNA synthesis. exhibited HIV-inhibitory activity	• Lin *et al.*, [67] • Semple *et al.*, [68] • Yu *et al.*, [69]

13	Terpenoids:
	• Parthenolide
	• Sesquiterpene
	• Triterpenoids
	  • Moronic acid
	  • Ursolic acid
	  • Maslinic acid
	  • Saponin
		Effect on HCV replication in a subgenomic RNA replicon assay system	HCV	Parthenolide is able to potentiate the interferon α-exerted anti-HCV effect.	Hwanga *et al.*, [70]

14	Polysaccharides Carrageenan	Effect of polysaccharides on viral replication	HSV-1	This sulfated polysaccharide inhibits a step in virus replication subsequent to viral internalization but prior to the onset of late viral protein synthesis.	González *et al.*, [71]
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	Significant prevention of formation of malignant foci by the polysaccharide at this late time was most likely due to its action against the subsequent secondary infection cycle.	Talyshinsky *et al.*, [72]
16	Alkaloids	Studies of the mechanism of action of michellamine B, a novel anti-human immunodeficiency virus (HIV) alkaloid Effects of Amaryllidaceae alkaloids and their derivatives upon herpes simplex virus (type 1)	HIV Herpes Simplex Virus (Type 1)	Michellamine B acts both at an early stage of the HIV life cycle by inhibiting RT as well as at later stages by inhibiting cellular fusion and syncytium formation. Antiviral effect could be partly explained as a blocking of viral DNA polymerase activity.	McMahon *et al.*, [73] Renard-Nozaki *et al.*, [74]

(Table 3. Contd….)

	Name of the Phytochemicals /Class	Details of Study	Virus	Mechanism	References
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.*, [75]
18	Olomoucine and Roscovitine	Potential applications of CKIs are being studied presently in viral diseases.	Cytomegalovirs and Herpes simplex virus	Cyclin dependant kinase inhibitor inhibits viral replication	• Bresnahan *et al.*, [76] • Schang *et al.*, [77]
19	Psoralen compounds • 4'-Hydroxymethyltrioxsalen • 4'-Aminomethyltrioxsalen	Effect on infectivity of DNA and RNA viruses, examined by immunofluorescence and radioimmunoassay and by measuring the capacity of the herpes simplex virus-infected cells to stimulate virus-specific lymphocyte proliferation.	Herpes Simplex Virus	The infectivity of the virus-infected cells could be totally eliminated without altering their viral antigenicity. Psoralen compounds covalently bind to nucleic acids when irradiated with long wavelength of UV light.	Redfield *et al.*, [78]
20	DNJ	Mechanism of antiviral Action of iminosugar derivatives against Bovine Viral Diarrhea Virus	Bovine Viral Diarrhea Virus	Effect has been attributed to the reduction of viral secretion due to an impairment of viral morphogenesis caused by the ability of DNJ-based iminosugar derivatives to inhibit ER α-glucosidases	Durantel *et al.* [79]
21	• Naturally occurring thiophene • Alpha-terthienyl (1) • 15 synthetic analogues	Evaluation of 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 analogues had significant toxicity, with minimum inhibitory concentrations in the range of 0.02-40 microM.	Marle *et al.* [80]
22	Gingerol	Common cold throat infection	Common Cold Virus	Improvement of NK cell lysing activity was demonstrated which are specific for the elimination of virus-infected cell and mutated cells. Positive effect on immune system	Chrubasik *et al.* [51]
23	Capsaicin	HSV infections	HSV	Interference with intraneuronal transport of virus	Stanberry *et al.*, [81]

(Table 3. Contd….)

	Name of the Phytochemicals /Class	Details of Study	Virus	Mechanism	References
24	Curcumin	Coxsackievirus infection	Coxsackievirus	Dysregulation of the UPS	Xiaoning *et al.*, [82]
25	Lutein / Zeaxanthin	HIV	HIV	Lowers oxidative stress in HIV patients, helps to restore metabolism	Dikici *et al.*, [83]

Poliomyelitis

Poliovirus, the causative agent of polio is a human enterovirus. Polio, short for poliomyelitis, is a disease that damages the nervous system and cause paralysis. The disease is normally prevalent in less developed Asian and African countries where polio immunization for kids is not very popular. However, inspite of the massive immunizationby the governments and non governmental organizations, polio remains a problem in many parts of the world. A large number of plant derived products have been evaluated for their activity against polio virus. Isoobtusitin (**61**), a prenylated coumarin showed substantial *in vitro* inhibitory activity against poliovirus (IC 50 = 2.9 μM) [85]. Isokaempferide (5,7,4'-trihydroxy 3-methoxyflavone) derived from *Psiadia* species was found to be inhibitor of poliovirus type 2 replication [96]. Tuli and his colleagues examined the antiviral action of 3-methyleneoxindole (MO), a plant metabolite, in HeLa cells infected with poliovirus. On the basis of data collected the 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 which already are bound to MO. This resulted in non recovery of virus-specific polysomes from infected cells treated with antiviral concentrations of MO [97].

Herpes

Herpes is caused by Herpes simplex virus 1 and 2 (HSV-1 and HSV-2 are strains of the herpes virus family) is a painful infection mainly affecting skin, eyes, mouth and genitals. There is no permanent cure for herpes but treatment can reduce the viral shedding. There have been efforts all around the globe to identify plant based treatment for this infection. Lyu *et al.* carried out anti-herpetic assays on 18 flavonoids in

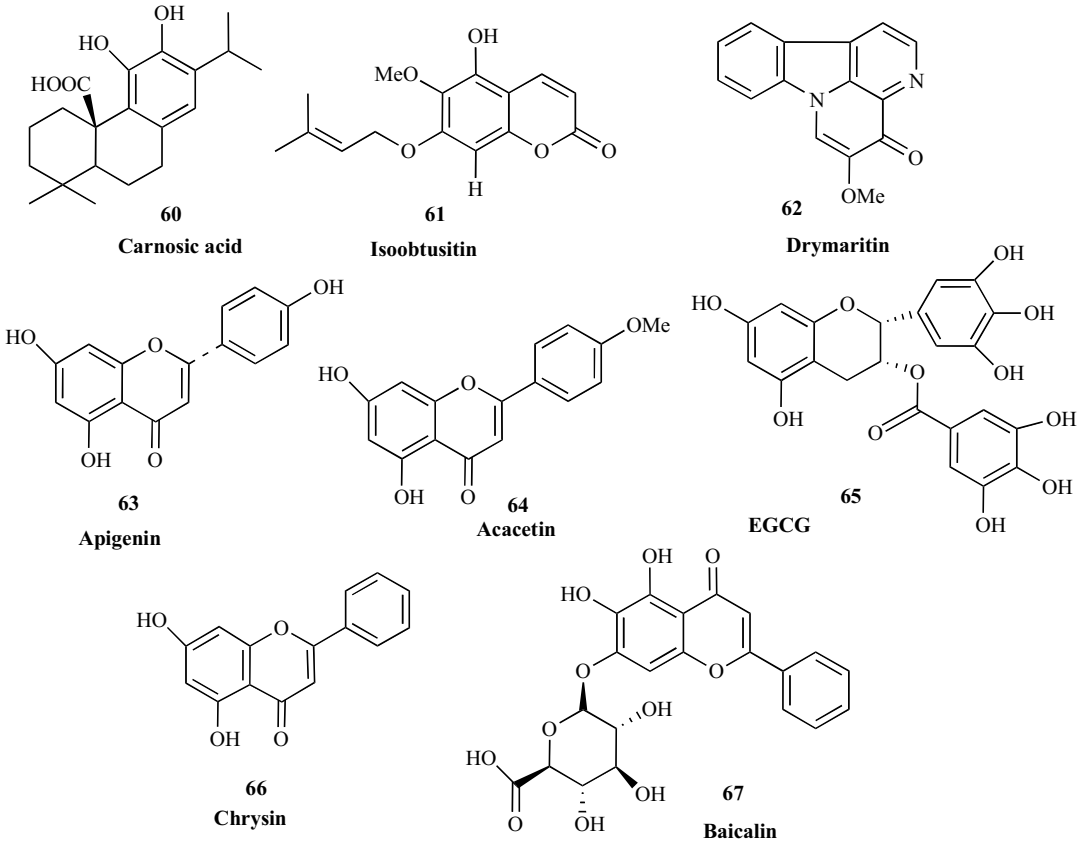


Fig. (11).

five classes and a virus-induced cytopathic effect (CPE) inhibitory assay, plaque reduction assay, along with yield reduction assay were performed [98]. EC, ECG, galangin and kaempferol exhibited strong antiviral activity whereas catechin, EGC, EGCG (**65**), chrysin (**66**), BA (**67**) showed moderate activity against HSV-1. Amongst 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 flava-nones naringenin expressed a strong inhibitory effect (5 μM [1.36 μg/mL]) against HSV-1. Similarly amongst 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, regarding compounds it was found that introduction of an a-orientated AcO group enhances the anti-viral activity [99]. Chattopadhyay and colleagues reported substantial anti-HSV activity of *Ophirrhiza nicobarica* extract at 300 μg ml-1. The alkaloid, flavonoid and β-sitosterol isolated from bioactive parts had a dose-dependent therapeutic efficacy, justifying their use [100]. Eugenol (4-allyl-1-hydroxy-2-methoxybenzene) was screened for efficacy against HSV-1 and HSV-2 viruses. It was was found in the *in vitro* experiments 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, 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. It is of interest to mention here that the eugenol-acyclovir combinations synergistically inhibited herpesvirus replication *in vitro* [101]. Nineteen compounds isolated from *Ranunculus sieboldii* and *Ranunculus sceleratus* were tested for inhibitory effects on hepatitis B virus (HBV) and Herpes simplex virus type-1 (HSV-1). The experiments revealed that apigenin 4'- O- alpha-rhamnopyranoside, apigenin 7- O-beta-glucopyranosyl-4'- O-alpha-rhamnopyranoside, tricin 7- O-beta-glucopyranoside, tricin, and isoscopoletin (**18**) possessed excellent antiviral activity against HBV replication. In addition protocatechuyl aldehyde (**19**) also displayed an inhibiting activity on HSV-1 replication [102]. 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 [103]. 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 unenveloped single-stranded RNA virus) at concentrations varying from 12-47/Lg/ml [104]. 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 replication of HSV-1 virus on herpes simplex virus type-1 (HSV-1) replication in vero cells [105]. Du *et al.* isolated flavonoid leachianone from root bark of *Morus alba* showing potent antiviral activity. A flavonoid moralbanone, having characterstic prenyl chain, along with seven other known compounds, was isolated from the root bark of *Morus alba* L. Amongst all the isolated compounds Leachianone G showed potent antiviral activity (IC50=1.6 μg/ml) [106]. 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 inhibition of replication on herpes simplex virus type-1 [107]. 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 Herpes simplex virus [108]. Flavonol glycosides (from quercetin andisorhamnetin) derived from stems of *Alphitonia philippinensis* have been reported to inhibit the replication of herpes simplex virus type-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 herpes simplex virus type-1 (HSV-1) replication in vero cells, epigallocatechin 3-O-gallate and samarangenin B exhibited potent inhibitory activities in HSV-1 replication. Comparison of the IC50 values indicated these both compounds had higher inhibitory activities than the positive control acyclovir (38.6 +/- 2.6 *vs*. 55.4 +/- 5.3 microM, $P < 0.001$; 11.4 +/- 0.9 *vs*. 55.4 +/- 5.3 microM, $P < 0.0005$). [109]. Three bis anthraquinones glucoside isolated from *Hypericum triquetrifolium* were evaluated for their antiviral activity against Herpes simplex virus 1. It was observed that Skyrin-8-O-beta-D-glucopyranoside exhibited antiviral activity against the DNA virus Herpes simplex type I. [110]. There are already reports in literature regarding regarding excellent anti-herpes simplex virus (HSV) activity of *Maclura cochinchinensis* in *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 micrograms/ml and 50.8 micrograms/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 micrograms/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 the free hydroxyl groups were required for anti-HSV-activity, as demonstrated previously by other workers for the antiviral activity of other flavonoids [111].

Hepatitis

Hepatitis derives its name from Greek words *hepato* and *itis* which literally stands for liver inflammation. There are several type of viral Hepatitis such as Hepatitis A, B,C,D,E,F,G . Hepatitis is also caused by mumps virus, rubella virus, 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 (IC 50 = 17.1 μg/mL) [85].) 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). 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 per cent (IC^sub 50^) of 100 and 50 μg/ml respectively. [112a]. Fractionationof the methanol extract of Angelica dahurica Benth et Hook resulted in the isolation of six furocoumarins including imperatorin Among these, compounds imperatorin (**72**) and (+)-byakangelicin exhibited strong hepatoprotective activities, displaying EC50 values of 36.6 +/- 0.98 and 47.9 +/- 4.6 □M, respectively [112b].

Constituents isolated from *Ranunculus sieboldii* and *Ranunculus sceleratus* were tested for inhibitory effects on hepatitis B virus (HBV) and Herpes simplex virus type-1 (HSV-1). It was shown that apigenin 4'-O-alpha-rhamnopyranoside, apigenin 7-O-beta-glucopyranosyl-4'-O-alpha-rhamnopyranoside, tricin 7-O-beta-glucopyranoside, tricin, and isoscopoletin possessed substantial inhibitory activity against HBV replication [113]. Ellagic acid (**73**) is isolated from *Phyllanthus urinaria* has exhibited 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 [114].

Influenza

The influenza virus is an RNA virus belonging to family *Orthomyxoviridae* is the causative organism for influenza commonly known as viral flu. There are three types of viruses known to cause influenza i.e.influenza virus A, influenza virus B and influenza virus C. As an integral part of traditional therapy in India and China plant extracts was routinely used to cure flu. A number of active biological compounds have have been found to possess excellent antiviral activity against influenza virus. Antiviral flavonoid 2"-O-(2'"-methylbutanoyl) isoswertisin obtained from flower of *Trollius chinensis* was found to be moderately active towards 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'"-methylbuta-noyl) isoswertisin and vitexin galactoside. In antiviral assays, compound was found to be moderately active towards influenza virus A [115]. 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 replication of these viruses in a dose-dependent manner. It was noticed that the agents suppressed replication of these viruses from 6 to 12 h after incubation in a dose-dependent manner by 50% at 20 micro M, 90 percent at 40 microM, respectively. Remarkably 5,7,4'-trihydroxy-8-methoxyflavone, at the concentration of (50 micro M) reduced the release of B/Ibaraki virus in the medium by 90-93% when it was added to the MDCK cells at 0 to 4 h after incubation. [116]. 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

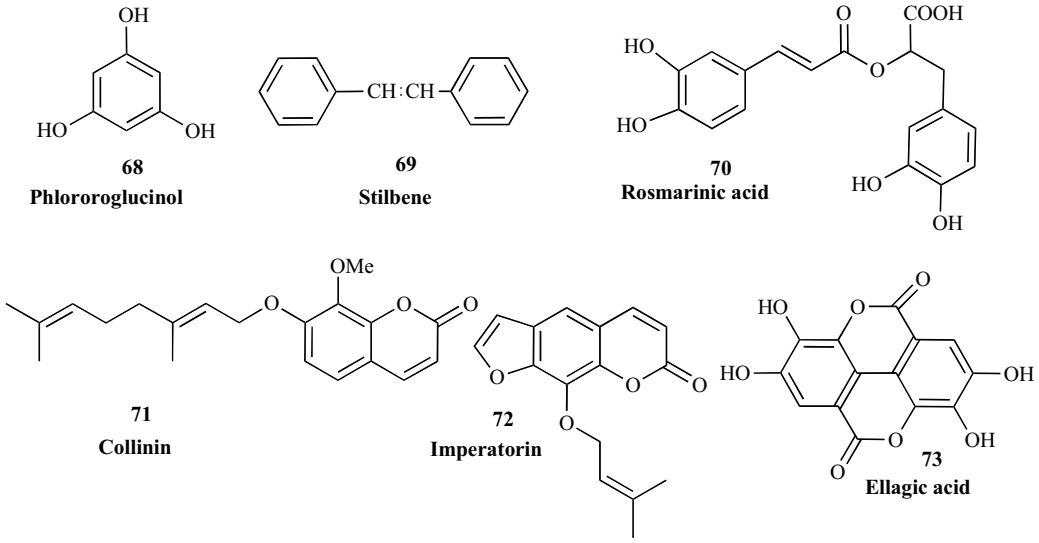


Fig. (12).

before or 4 h after virus exposure, only lung consolidation was significantly reduced. [117].

Common Cold

Rhinovirus, (derived from the Greek word rhin- denoting nose) the main causative agent of common cold belongs to genus of the *Picornaviridae* family. Since ancient times, plants extract have been used to cure common cold especially in the Indian subcontinent. Several homo-isoflavonoids, chloro-substituted rac-3-benzylchroman-4-ones were evaluated for *in vitro* activity against selected picornaviruses by means of the plaque reduction assay. The antiviral activity of the isomers and their racemates was examined against human rhinovirus serotype 1B and 14 infections. All homo isoflavonoids tested exhibited an inhibitory effect on rhinovirus replication with an activity depending on virus serotype and compound [118a]. The viral respiratory infections which are caused by picornaviruses have significant consequences in both children and adults. The infection result in producing exacerbations of asthma and other pulmonary disorders besides other respiratory tract abnormalities. Douglas and colleagues have reported antiviral activity of Vitamin C against rhinovirus [118b]. In an another report plants derived from family Echinacea (family *Asteraceae*) have been shown useful for preventing and treating the common cold [118c]. 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. The compounds were found to be interfering 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 all 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's worth mentioning that majority of derivatives were found to be effective against serotype 14, often with a higher potency [119].

Multiple Targets

Considerable large number of studies have 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 i.e. diallyl disulfide (**37**), diallyl thiosulfinate (**39**) (allicin), allyl Me thiosulfinate (**74**), ajoene (**75**), alliin (**76**), deoxyalliin (**77**), and diallyl trisulfide [120].

In an effort to determine the mechanistic action of garlic compounds to explain their antiviral action direct pre-infection incubation assays was used, to determine the *in vitro* virucidal effects against selected viruses including, herpes simplex virus type 1, herpes simplex virus type 2, parainfluenza virus type 3, vaccinia virus, vesicular stomatitis 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 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 an 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 [120]. Singh *et al.* have investigated interaction between chemokine receptor CXCR4 and flavonoids using in silico docking studies. On basis of their studies it was 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 [120b]. The moderate antiviral activity of the mixture of quercetin 3-O-beta -glucoside and quercetin 3-O-beta-galactoside derived from *Chamaesyce thyliamifo* against HSV-1 and BVDV viruses was also reported. [121]. 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 human rhinovirus (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 [122]. 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 herpesviruses (HSV-1, HSV-2) and adenoviruses (ADV-3, ADV-8, ADV-11). Results showed that 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). [123]. In last decade a there has been a lot of focus on the imino sugar glucosidase inhibitors have selective antiviral activity against certain enveloped, mammalian viruses [124a]. 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 modification of the alkyl side chain could influence antiviral activity [124 b]. De Almeida *et al.* reported strong inhibition of an infusion of *Persea americana* leaves against herpes simplex virus type 1 (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), Au-

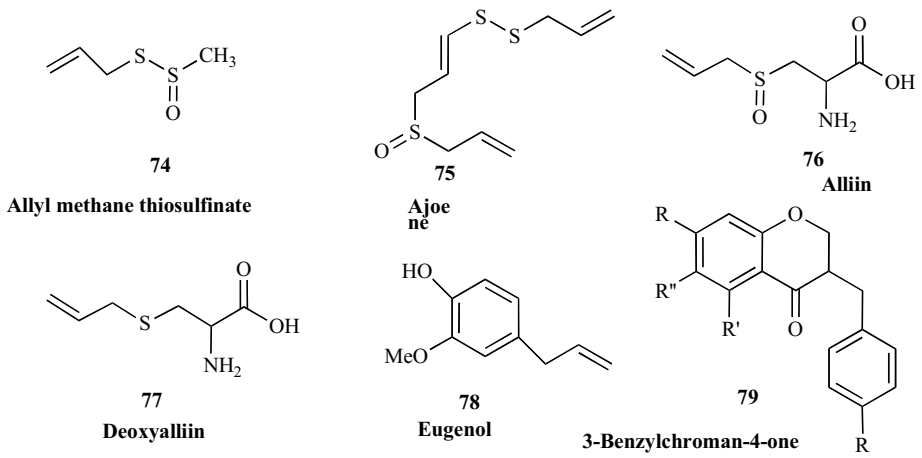


Fig. (13).

jeszky'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. [125].

Miscellaneous

There exists huge volume of literature regarding the evaluation of plant derived compounds against several other viral targets apart from the ones listed above. Miyake and colleagues reported excellent activity of 8-geranyloxypsoralen (**80**) (isolated in low yields from *Citrus Limon* against tumor promoter TPA-induced Epstein-Barr virus activation in Raji cells. It was shown that at a concentration of 10 μM, the inhibitory activity was 79.3% [87]. Resveratrol (**81**) was found to inhibit varicella-zoster virus (VZV) replication in a dose-dependent and reversible manner. It was further demonstrated that through real-time RT-PCR studies that protein and mRNA levels of IE62, an essential early viral protein, were reduced when compared to controls. The drug effectively limited VZV replication if added during the first 30 hours of infection [126]. Chalcone has been regarded as major active template amongst flavonoids for its antiviral activity. Cyclohexenyl chalcone derivatives 4-hydroxypanduratin A and panduratin derived from *Boesenbergia rotunda* displayed substantial inhibitory activities towards dengue 2 virus NS3 protease (Ki values of 21 and 25muM, respectively). The authors showed that the interaction between the NS2B cofactor and NS3pro was important not only for the correct fold of the protease but also for the correct interaction with the substrate [127]. The inhibitory effects of some flavonoids on the infectivity of rotavirus causing sporadic diarrhea in infants was evaluated. It was reported that diosmin (**82**) and hesperidin (**83**) had most potent activity on rotavirus infection [128]. Baicalin (BA) is a well known flavonoid compound purified from the medicinal plant *Scutellaria baicalensis* and has been reported to possess anti-inflammatory and anti-viral activities. As a part of mechanistic study of the action of BA, experiments were conducted to find out if BA could interfere with chemokines or chemokine receptors, which are considered to be critical mediators of inflammation and infection. BA inhibited the binding of a number of chemokines to human leukocytes or cells transfected to express specific chemokine receptors. [129].

In an effort to shed light on the mechanistic details of antiviral activity of 3-methyl quercetagetin it was reported that the flavonoid had significant activity against tomato bushy stunt virus which was attributed to the interference of the flavonoid during the virus infection initiation [130]. Antiviral activities of seven flavonoids belonging to kaempferol series were evaluated against human HCMV. Flavonoids bearing acyl groups were more active [131]. 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 0.2mL 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 in chronically infected mice treated freshly prepared crude extract of *Chelidonium majus* ($p = 0.0057$ and $p < 0.001$) [132]. The possible antiviral effect of flavonoids obtained from *Tephrosia madrensis, Tephrosia viridiflora* and *Tephrosia crassifolia* on dengue viruses was evaluated by Sanchez and colleagues who concluded that glabranine and 7-O-methylglabranine presented 70% inhibition on the dengue virus [133]. Major clinical trials relating to the antiviral activities of phytochemicals have been summarized in Tabe **4**.

CONCLUSIONS AND FUTURE DIRECTION

As already discussed numerous epidemiological and experimental studies have shown that phytochemicals are active in various *in vivo* and *in vitro* antiviral studies. However there are several issues to be addressed before these phytochemicals can actually be used in treatment of viral diseases.

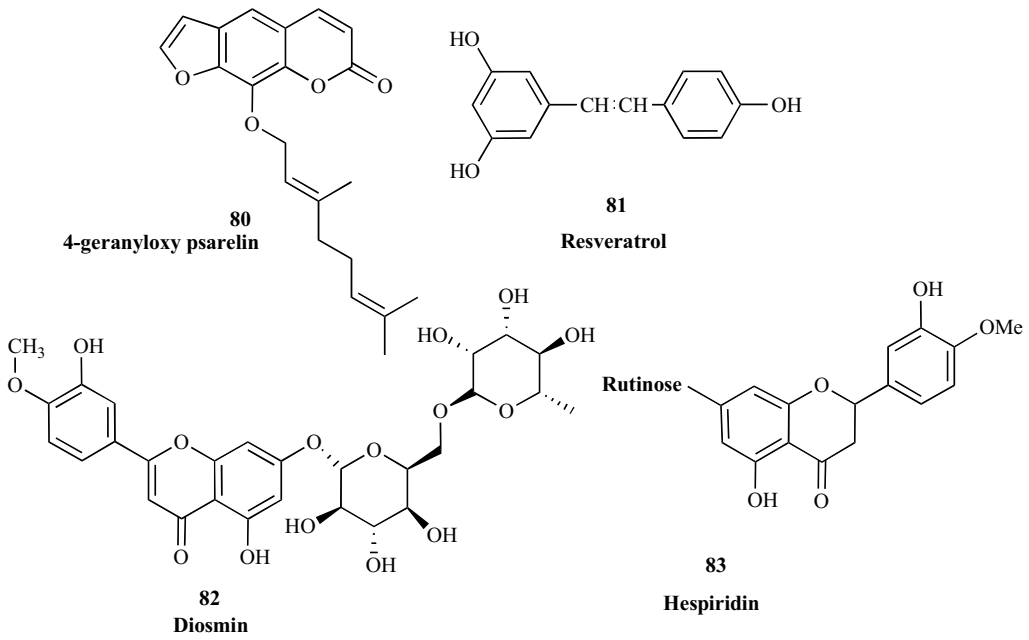


Fig. (14).

Scientists from different interdisciplinary area are routinely investigating new and newer plant products for antiviral activities but the fact remains that only one percent of the plants have been exploited. With the current rate of destruction of natural resources by modern civilization we stand chance of losing the invaluable resources until suitable corrective methods are taken.

The fact remains that the currently available orthodox drugs cannot eliminate all or even most of viral diseases. It is therefore, of great importance to develop new medicinal plant products vital in controlling the threats posed by some pathogenic viruses. Since most of the current studies were performed *in vitro*, it is important that their results be further extended to *in vivo* animal and human studies. One of the major weaknesses of the existing research is that most of the candidates that made up to clinical trials do not have high methodological quality. Multi-centric large scale randomized clinical trial (RTC) could be used along with rigorous independent trials. In order to avoid inconsistency in the experimental results of antiviral studies it would be beneficial to standardize the extraction and testing antiviral compounds. Lack of standardization of dosage, schedule etc has lead to many inconsistencies in results.

Design and proper rigorous execution to get meaningful data is essential. Since many assays are not standardized results are not comparable. It would be advantageous to standardize the isolation method to make things more systematic. Efforts should be directed to keep record of safety and effectiveness of phytochemical treatment that is generally uncontrolled. Insufficient methods are available to measure the oxidative damage, *in vitro* and *in vivo*. There is dire need to improve the analytical techniques in order to collect more data. Modified techniques need to be developed to collect data on absorption metabolism and excretion. Bioavailability in fact limited information is available on the absorption of the agent. There is still no definite data if glycosylation facilitates absorption or not. The lack of *in vivo* testing in different biological assays has let to practically no contribution of flavonoids in treating patients with viral infections.

Optimization of phytochemicals through structure modification may allow for the development of acceptable set of agents. Interaction between the antimicrobial agents and the target sites should be characterized so as to obtain information for alteration of structure. The screening of bioactive substances along with the determination of structure activity relationship is essential for coming out with more effective drug against infective agent. A carefully planned strategy may yield antiviral compounds with desired potency with less untoward side effects. Cohort studies and controlled intervention trials with focus on specific viral target and specific population should be conducted. Synergistic, additive or inhibitory effects of various phytochemicals should be addressed. Most of the antiviral studies have revolved around compounds readily available in nature. Efforts should be directed to synthesize analogs keeping in mind the fundamental biochemistry and metabolic pathway of compounds. Dietary mechanisms studies may be hard to interpret. These studies are labor intensive and consuming long time. It is essential that the participants sample size may be big enough so that statistical conclusions can be made. As is true with other plant derived agents there is complication to determine optimum intake. The quantity used *in vivo and in vitro* studies in animals cannot be translated for use by human since the amounts become unrealistic. The data on the absorption metabolism and the excretion of phytochemicals in humans is contradictory and scarce. It should be recognized that the study of phytochemicals in humans is complex because of very less information is available on bioavailability. Immense diversity in growth condition processing cooking and harvest etc. the amount of naturally active agents can vary.

Literature survey reveals that there is no well-documented scientific data on the ability of herbal products.

Table 4. Clinical Trials

	Study Details/ Condition Treated	Agent	Results	References
1	Chronic hepatitis B	*Phyllanthus amarus*	Substantially cleared hepatitis B surface antigen	Thyagarajan *et al.* [134]
2	Chronic hepatitis B	*Phyllanthus amarus*	Significantly cleared hepatitis B surface antigen	Thyagarajan *et al.* [135]
3	Chronic hepatitis B	*Phyllanthus amarus*	No significant changes in levels of HbsAg	Thamlikitkul *et al.* [136]
4	Chronic hepatitis B	*Phyllanthus amarus*	No changes in levels of HBsAg, HbeAg, HBV DN	Berk *et al.* [137]
5	Chronic hepatitis B	*Phyllanthus amarus*	Significant reduction in HbeAg in treated group	Zhang *et al.* [138]
6	Chronic hepatitis B	*Phyllanthus amarus*	No significant changes in levels of HbsAg, HbeAg	Miln *et al.* [139]
7	Chronic hepatitis B	*Phyllanthus amarus*	No significant changes in levels of HbsAg, HbeAg	Peng *et al.* [140]
8	Chronic hepatitis B	*Phyllanthus amarus*	Significant reduction in HbeAg in treated group	Huang *et al.* [141]
9	Chronic hepatitis B	*Phyllanthus urinaria*	Significant reduction in HbeAg in treated group	Zhu *et al.* [142]
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.* [143]
11	Chronic hepatitis B	*Phyllanthus urinaria*	Significant reduction in HBV DNA in treated group, Improvement in liver function	Huang *et al.* [144]
12	Chronic hepatitis B	*Phyllanthus Amarus, Phyllanthusn niruri, Phyllanthus urinaria*	Improvement in patient condition *Phyllanthus urinaria* specifically effective; no side effect	Wang *et al.* [145]
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.* [146]
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.* [147]
15	Hepatitis B virus	*Phyllanthus amarus*	Failed to inhibit B surface antigen in patient with hepatitis B virus	Doshi *et al.* [148]
16	Chronic hepatitis C	*Iscador (Viscum album extract)*	Substantial decrease in HCV production	Tusenius *et al.* [149]

(Table 4. Contd….)

	Study Details/ Condition Treated	Agent	Results	References
17	Chronic hepatitis C	*Glycyrrhiza glabra*	Mean decrease in ALT levels 26% in treated group, 6% in placebo	van Rossum *et al.* [150]
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.* [151]
19	Hepatitis C	*Glycyrrhiza glabra*	Mean change in ALT levels 47%	van Rossum *et al.* [152]
20	Hepatitis C	*Phyllanthus niriri*	Healing complete by day 8 in 96% (natural recovery usually 10–14 days)	Mehrotra *et al.* [153]
21	Hepatitis C patients	Phlogenzym, a combination of hydrolytic enzymes with the flavonoid rutosid	Phlogenzym was found to be even superior to ribavirin and interferon (established medication). The tolerance of the oral enzymes was excellent.	Stauder *et al.* [154]
22	Chronic hepatitis	*Glycyrrhiza glabra*	Significant overall improvement in clinical markers and in some liver function tests	Suzuki *et al.* [155]
23	Chronic viral hepatitis	*Glycyrrhiza glabra*	Greater improvement ALT levels in Group A than Group B (P=0.0002)	Miyake *et al.* [156]
24	Alcoholic or non-alcoholic chronic hepatitis.	Silybin/phosphatidyl coline complex	A statistically significant drop (P<.01-.001) in ALT and GGT occurred at doses of 240 mg or more	Vailaii *et al.* [157]
25	Jaundice in HBV persons	*Phyllanthus amarus*	No significant intergroup differences	Narendranathan *et al.* [158]
26	Liver cirrhosis	*Silymarin*	Silymarin has no effect on survival	Peres *et al.* [159]
27	Liver cirrhosis	*Silymarin*	Indicated that treatment was effective in patients with alcoholic cirrhosis	Ferenci *et al.* [160]
28	Influenza A	*Sambucus nigra L*	Disappeared 4 days earlier in treated group	Thom *et al.* [161]
29	Influenza B	*Sambucus nigra L*	Significantly faster recovery in treated group	Zakay-Rones *et al.* [162]
30	Common cold	*Andrographis paniculata*	Visual analogue scale, Assessment of symptoms, Days sick leave Significant reduction in symptoms and in days sick-Leave in treated group	Melchior *et al.* [163]
31	Common cold	*Andrographis paniculata*	Significant reduction in intensity of symptoms in treated group	Caceres *et al.* [164]
32	Common cold	*Andrographis paniculata*	Reduced symptoms and faster recovery in treated group	Hancke *et al.* [165]
33	Common cold (prevention and treatment)	*Allium sativum L*	Significantly fewer colds of shorter duration in treated group	Josling *et al.* [166]

(Table 4. Contd….)

	Study Details/ Condition Treated	Agent	Results	References
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.* [167]
35	Chronic HCV patients	*Silymarin* Capsules	Well tolerated patients improved over time	Tanamly *et al.* [168]
36	Chronic HCV	*Silymarin*	No visible effect	Gordon *et al.* [169]
37	Chronic HCV	*Silymarin* capsules plus antioxidants and vitamins herbals	Well tolerated 48% patients positive response	Melham *et al.* [170]
38	HCV patients	*Silymarin*	No adverse effect but no effect on outcome	Strickland *et al.* [171]
39	Patients with detectable HCV RNA	*Silymarin* extract	Non interferon based standard therapy better than silymarin	El-Zayadi *et al.* [172]
40	HSV viral infection	*Melissa officinalis*	Treament effective without any side cytotoxic side effects	Koytchev *et al.* [173]
41	Herpes simplex infection <72 h duration	*Melissa officinalis L*	Significant reduction in symptom score in treated group on day 2	Wöbling *et al.* [174]
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.* [175]
43	Genital herpes	*Aloe vera*	Mean days to healing, number of patients cured Significantly shorter mean time to healing and cured patients at 2 weeks in treated group.	Syed *et al.* [176]
44	Genital herpes	*Clinacanthus nutans*	*Phyllanthus niruri*, has anti-HBsAg activity.	Jayavasu *et al.* [177]
45	Recurrent herpes labialis	*Melaleuca alternifolia*	Time to lesion healing No significant inter group differences	Carson *et al.* [178]
46	Herpes labialis <24 h	*Salvia officinalis*	No significant intergroup differences	Saller *et al.* [179]
47	Herpes zoster	*Clinacanthus nutans*	Lesion healing significantly faster in treated group	Sangkitporn *et al.* [180]
48	Herpes zoster	*Clinacanthus nutans*	Complete healing, lesion healing significantly faster in treated group	Charuwichitra *et al.* [181]
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.* [182]

(Table 4. Contd….)

	Study Details/ Condition Treated	Agent	Results	References
50	HIV	*Buxus sempervirens*	Significant delay of progression to disease	Durant *et al.* [183]
51	HIV	*Glycyrrhiza glabra*	Some improvement in asymptomatic carriers, none in AIDS patients	Gotoh *et al.* [184]

The study of plant product is complex because of the vast number of agents and the scarcity of bioavailability data. Herbal studies are often published in journals, which are not readily available. In addition there is a tendency not to publish negative results which might not be a good one. Many times identical natural products are tested by many different groups with negative outcomes. If negative results were already known efforts will be directed to find new herbal agents.

Understandably there is lack of interest of large multinationals to invest on herbal medicines because of non-clarity of intellectual property rights. In this case scenario government agencies and nongovernmental organizations should join hands to determine the effectiveness of botanicals. A very aggressive strategy to include people with indigenous knowledge, nongovernmental organization, chemists, microbiologists and clinicians would be of great help. Otherwise we stand a chance of losing several untapped resources due to extinction of plants. Efforts should be dedicated to determine the minimum quantity of the active compounds. Biochemistry of metabolism and potential toxicity of different agents needs to be explored. In addition to the structural alternation of active antiviral agents investigation of mechanistic action may be a productive area of research. If these gaps are covered we might get close to developing a new class of drugs that work on different target sites to those in current use. It can also lead to striking a balance between the toxicity and the activity of a particular agent and the interaction of particular agent with target site could give lead to design and synthesis of novel antiviral agent.

ABBREVIATIONS

HRV = Human rhinovirus

PV = Polio virus

HCMV = Human cytomegalovirus

HSV = Herpes Simplex Virus

BVDV = Bovine viral diarrhea virus

BA = Baicalin

BCA = Baicalein

UPS = Ubiquitin- proteasome system

TI = Therapeutic index

CMV = Cytomegalovirus

NDGA = Nordihydroguaiaretic acid

SARS = Severe acute respiratory syndrome

PC = Polyphenolic complex

MuSV = Murine sarcoma virus

MuLV = Retroviruses murine leukemia virus

BGM cells = Buffalo Green Monkey cells

HBsAg = hepatitis B surface antigen)

HbeAg = Hepatitis 'e' antigen

AST = Aspartate aminotransferase

ALT = Alanine aminotransferase

GGT = Gamma-glutamyl Transferase

RTC = Randomized clinical trial

IgA = Immunoglobulin A

IgG = Immunoglobulin G

IgM = Immunoglobulin M

DNAP = Deoxyribonucleic acid fraction

CPE = Cytopathic effect (CPE)

AMV = Avian Myeloblastosis

MMLV = Maloney Murine Leukemia Virus

RT = Reverse transcriptases

RAV-2 = Rous-associated virus-2

EGCG = Epigallocatechin gallate

ECG = Epicatechin gallate

EGC = Epigallocatechin

REFERENCES

[1] International Symposium on Medicinal Plants, April 19-21, **1993**, in Philadelphia, U.S.A The Morris Arboretum and the World Health Organization (WHO).

[2] Wenzel, U.; Kuntz, S.; Daniel, H.J. *Pharmacol. Exp. Ther*., **2001**, *299*, 351.

[3] Pietta, P.; Gardana, C.; Pietta, A. In: *Flavonoids in Health and Disease*; C. Rice-Evans,C.; Packer, L. Ed.; Marcel Dekker Inc: New York/Basel, **2003**, 43-70.

[4] Tsuchiya, Y.; Shimizu, M.; Hiyama, Y.; Itoh, K.; Hashimoto, Y.; Nakayama, M.; Horie, T.; Morita, N. *Chem. Pharm. Bull.,* **1985**, *33*, 3881.

[5] Selway, J.W.T. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure–Activity Relationships*, Cody, V.; Middleton, E.; Harborne, J.B. Eds.; Alan R. Liss, Inc: New York, **1986**, 521-526

[6] de Bruxelles, G.L.; Roberts, M.R. *Plant Sci.*, **2001**, *20*, 487.

[7] Jassim, S.A.A.; Naji, M.A. *J. Appl. Microbiol.*, **2003**, *95*, 412.

[8] Abad, M.J.; Guerra, J.A.; Bermejo, P.; Irurzun, A.; Carrasco, L. *Phyto. Res.*, **2000**, *14*, 604.
[9] Martin, K.W.; Ernst, E. *Antivir. Ther.*, **2003**, *8*, 77.
[10] Havsteen, B. *Biochem. Pharmacol.*, **1983**, *32*, 1141.
[11] Watkins, T.; Resch, W.; Irlbeck, D.; Swanstrom, R. *Antimicrob. Agents Chemother*., **2003**, *47*, 759.
[12] Rose, R.E.; Gong, Y.F.; Greytok, J.A.; Bechtold, C.M.; Terry, B.J.; Robinson, B.S.; Alam, M.; Colonno, R.J.; Lin, P.F. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 1648.
[13] Balfour, H.H. *The New Eng. J. Med.*, **1999,** *340*, 1255.
[14] Kuper, H.; Adami, H.-O.; Trichopoulos, D. *J. Int. Med.*, **2000**, *248*, 171.
[15] (a) Thomas, P.R.S.; Nash, G.B.; Dormandly, J.A. *Br. Med. J.,* **1988**, 296, 1673. (b) Loewenstein, W.R. *Biochem. Biophys. Acta,* **1979**, *560,* 1. (c) Gerdin, B.; Srensso, E. *Int. J. Micro. Cir. Clin. Exp.,* **1983**, *2,* 39. (d) Deng, J.; Kelley, J.A.; Barchi, J.J.; Sanchez, T.; Dayam, R.; Pommier, Y.; Neamati, N. *Bio. Med. Chem.*, **2006**, *14*, 3785 (e) Nowakowska, Z. *Eur. J. Med. Chem.*, **2007**,*42*, 125.
[16] Phrutivorapongkul, A.; Lipipun, V.; Ruangrungsi, N.; Kirtikara, K.; Nishikawa, K.; Maruyama, S.; Watanabe, T.; Ishikawa, T. *Chem. Pharm. Bull.*, **2003**, *51*, 187.
[17] Likhitwitayawuid, K.; Chaiwiriyaa, S.; Sritularaka, B.; Lipipunb, V. *Chem. Biodivers.*, **2006**, *3*, 1138.
[18] (a) Prendergast, P.T.US Patent **2003**, Patent No 6555523 (b) Santhosh, C.; Mishra, P. C. *Indian J. Biochem. Biophys*. **1996**, *33,* 458.
[19] Moriarty, R.M.; Surve, B.C.; Naithani, R.; Chandersekera, S.N.; Tiwari, V.; Shukla, D., Synthesis and antiviral activity of Abyssinone II analogs., *Abstracts of Papers*, 233rd ACS National Meeting, Chicago, IL, United States, March 25-29, **2007**
[20] Vlietinck, A.J.; Bruyne,T.D.; Vandenberghe, D.A. *Curr. Org. Chem.;* **1997** *1,* 307.
[21] Kaul, T.N.; Middleton Jr. E.; Pearay, M.D.; Ogra, L. *J. Med. Vir.,* **2005,** *15, I(1),* 71.
[22] Twaij, H.A.A.; Sayed-Ali, H.M.,; AlZohry, A.M. *J. Biol. Sci. Res*., **1988,** *19(1),* 41.
[23] Andersen, O. M.; Helland, D. E.; Andersen, K. J. *PCT Int. Appl*., **1997**, 121.
[24] Takatsuki, A.; Tamura, G.; Arima, K. *App. Micro*., **1969**, *17*, 825.
[25] Mashava, P. *PCT Int. Appl*., **1996**, 38.
[26] Conti, C.; Orsi, N.; Stein, M.L. *Antiviral Res*., **1988**, *10*, 117.
[27] Yu, D.; Suzuki, M.; Xie, L.; Morris-Natschke, S.L.; Lee, K.-H. *Med. Res. Rev.*, **2003**, *23*, 322.
[28] Creagh, T.; Ruckle, J.L.; Tolbert, D.T. *Antimicrob. Agents Chem.*, **2001**, *45*, 1379.
[29] Li, Y.; Leung, K.T.; Yao, F.; Ooi, L.S.M.; Ooi, V.E.C. *J. Nat. Prod.*, **2006**, *69,* 833.
[30] Charles, L.; Laure, F.; Raharivelomanana, P.; Bianchini, J.P. *J. Mass Spectrom.*, **2005**, *40*, 75.
[31] Farnsworth, N.R. *J. Pharm. Sci.*, **2006**, *55(3*), 225 (b) Houghton, P.J; Woldemariam, T.Z; Khan A.I.; Burke, A.; Mahmood, N. *Antiviral Res.,* **1994**, *25(3-4),* 235.
[32] Wen, C.-C.; Kuo, Y.-H.; Jan, J.T.; Liang, P.H.; Wang, S.Y.; Liu, H.G.; Lee, C.K.; Chang, S.T.; Kuo, C.J.; Lee, S.S.; Hou, C.C.; Hsiao, P.W.; Chien, S.C.; Shyur, L.F.; Yang, N.S. *J. Med. Chem.,* **2007**, *50,* 4087.
[33] Melikian, G.; Mmiro, F., Ndugwa, C., Perry, R., Jackson, J. B., Garrett, E., Tielsch, J.; Semba, R. D. *Nutrition*, **2001,** *17*, 567.
[34] Heldreth, B.; Turos, E. *Curr. Med. Chem.*, **2005**, *4*, 295 (b) Hunter, R.; Caira, M.; Stellenboom, N. *Nat. Prod. Mol. Ther.*, **2005,** *1056*, 234.
[35] Jariwalla, R.; Harakeh, S. In Subcellular Biochemistry: Ascorbic Acid: *Biochem. Biomed. Cell Biol.*, Harris, J.R., Ed.; NY: Plenum Press, **1996**, *25*, 215. (b) Andreone, P.; Fiorino, S.; Cursaro, C.; Gramenzi, A.; Margotti, M.; Di Giammarino, L.; Biselli, M.; Miniero, R.; Gasbarrini, G.; Bernardi, M. *Antiviral Res*., **2001**, *49*, 75.
[36] Cermelli, C.; Vinceti, M.; Scaltriti, E.; Bazzani, E.; Beretti, F.; Vivoli, G.; Portolani, M. *J. Trace Elem. Med. Biol.*, **2002**, *16*, 41 (b) H. Wójtowicza,H.; Kloca, K.; Maliszewskaa, I.; Młochowskia, J.; Piętkaa, M. ; Piasecki, E. *Farmaco,* **2004**, *59*, 863.
[37] La Colla, P.; Tramontano, E.; Musiu, C.; Marongiu, M.E.; Novellino, E.; Greco, G.; Massa, S.; Di Santo, R.; Costi, R.; Marino, A. *Antiviral Res.*, **1998**, *7*, A57.
[38] Botelho, M.V.; Orlandi, J.M.; de Melo, F.L.; Mantovani, M.S.; Linhares, R.E.; Nozawa, C. *Lett. Appl. Microbiol*., **2004**, *39*, 174.
[39] Ishihara, C.; Yoshimatsu, K.; Tsuji, M.; Arikawa, J.; Saiki, I.; Tokura, S.; Azuma, I. *Vaccine*, **1993**, *11*, 670.
[40] Liu, K.C.; Lin, M.T.; Lee, S.S.; Chiou, J.F.; Ren, S.; Lien, E.J. *Planta Med*., **1999**, *65*, 43.
[41] Hudson, J.B. *Antiviral Compounds from Plants*, CRC Press: Boca Raton, FL, **1990**.
[42] Wat, C.K., Biswas, R.K.; Graham, E.A.; Bohm, L.; Towers, G.H.; Waygood, E.R. *J. Nat. Prod.*, **1979**, *42*, 103.
[43] Neushul, M. *Hydrobiologia*, **1990**, *205*, 99.
[44] Gerber, P.; Dutcher, J.D.; Adams, E.V.; Sherman, J.H. *Proc. Soc. Exp. Biol. Med.*, **1958**, *99*, 590.
[45] Witvrouw, M.; DeClerq, E. *Gen. Pharm*., **1997**, *29*, 497.
[46] Huleihel, M.; Ishanu, V.; Tal, J.; Arad, S.M. *J. Appl. Phycol.*, **2001**, 13, 127.
[47] Konigheim; B.S.; Goleniowski, M.E.; Contigiani, M.S. *Drug Design Rev.*, **2005**, *2*, 81.
[48] Wang, C.Y.; Sun, S.W.; Lee, S.S. *Planta Med.*, **2004**, *70*, 1161.
[49] Ho, T.Y.; Wu, S.L.; Chen, J.C.; Li, C.C.; Hsiang, C.Y. *Antiviral Res*., **2007**, *74*, 92.
[50] Semple, S.J.; Pyke, S.M.; Reynolds, G.D.; Flower, R.L.P. *Antiviral Res.,* **2001**, *49*, 169.
[51] Chrubasik S; Pittler M.H; Roufogalis, B.D. *Phytomedicine*, **2005**, *12*, 684.
[52] Singh, D.P.; Moore, C.A.; Gilliland, A.; Carr, J.P. *Mol. Plant Pathol.*, **2004**, *5*, 57
[53] Ishitsuka, H.; Ohsawa, C.; Ohiwa, T.; Umeda, I.; Suhara, Y. *Antimicrob. Agents Chemother.,* **1982**, *22*, 611.
[54] Serkedjieva, J., A. *Phytother. Res.*, **1996**, *10*, 441.
[55] Malhotra, B.; Onyilagha, J.C.; Bohm, B.A.; Towers, G.H.N.; James, D.; Harborne, J.B.; French, C.J. *Phytochemistry*, **1996**, *43*, 1271.
[56] Li, B.Q.; Fu, T.; Dongyan, Y.; Mikovits, J.A.; Ruscetti, F.W.; Wang, J.M. *Biochem. Biophys. Res. Commun.*, **2000**, *276*, 534.
[57] Eversa, D.L.; Chaoa, C.-F.; Wanga, X.; Zhanga, Z.; Huonga, S. M.; Huang, E.S. *Antiviral Res.*, **2005**, *68*, 124.
[58] Castrillo, J.L.; Carrasco, L. *J. Virol*., **1987**, *61*, 3319.
[59] Takechi, M.; Tanaka, Y. *Planta Med.*, **1981**, *42*, 69.
[60] Sydiskis, R.J.; Owen, D.G.; Lohr, J.L.; Rosler, K.H.; Blomster, R.N. *Antimicrob. Agents Chemother.,* **1991**, *35*, 2463.
[61] Kurokawa, M.; Hozumi, T.; Basnet, P.; Nakano, M.; Kadota, S.; Namba, T.; Kawana, T.; Shiraki, K. *J. Pharmacol. Exp. Ther.*, **1998**, *284*, 728.
[62] Liu, K.C.; Lin, M.T.; Lee, S.S.; Chiou, J.F.; Ren, S.; Lien, E.J. *Planta Med.*, **1999**, *65*, 43.
[63] Bettega, J.M.R.; Teixeira, H.; Bassani, V.L.; Barardi, C.R.M.; Simões, C.M.O. *Phytother. Res*., **2004**, *18*, 819.
[64] Spedding, G.; Ratty, A.; Middleton, E. *Antiviral Res*., **1989**, *12*, 99.
[65] Salvati, A.L.; De Dominicis, A.; Tait, S.; Canitano, A.; Lahm, A.; Fiore, L. *Antimicrob. Agents Chemother*., **2004**, *48*, 2233.
[66] Song, J.-M.; Lee, K.-H.;.Seong, B.-L. *Antiviral Res.*, **2005**, *68*, 66.
[67] Lin, Y.M.; Flavin, M.T.; Schure, R.; Chen, F.C.; Sidwell, R.; Barnard, D.L.; Huffman, J.H.; Kern, E.R. *Planta Med.*, **1999**, *65*, 120.
[68] Semple, S.J.; Nobbs, S.F.; Pyke, S.M.; Reynolds, G.D.; Flower, R.L. *J. Ethnopharmacol.*, **1999**, *68*, 283.
[69] Yu, Y.B.; Miyashiro, H.; Nakamura, N; Hattori, M.; Cheol, P. J. *Arch. Pharm. Res.,* **2007**, *30*, 820.
[70] Hwanga, D.-R.; Wua, Y.-S.; Changa, C.-W.; Lienа, T.-W.; Chena, W.-C.; Tanb, U.-K.; Hsua, J.T.A.; Hsieh, H.P. *Bioorg. Med. Chem.*, **2006**, *14*, 83.
[71] González, M.E.; Alarcón, B.; Carrasco, L. *Antimicrob. Agents Chemother*., **1987**, *31*, 1388.
[72] Talyshinsky, M.M.; Souprun, Y.Y.; Huleihe, M.M. *Cancer Cell Int*., **2002**, *2*, 8.
[73] McMahon, J.B.; Currens, M.J.; Gulakowski, R.J.; Buckheit, Jr, R.W.; Lackman-Smith, C.; Hallock, Y.F.; Boyd, M.R. *Antimicrob. Agents Chemother*., **1995**, *39*, 484.
[74] Renard-Nozaki, J.; Kim, T.; Imakura, Y.; Kihara, M.; Kobayashi, S. *Res. Virol.*, **1989**, *140*, 115.
[75] Konigheim, B.S.; Goleniowski, M.E.; Contigiani, M.S. *Drug Des. Rev.*, **2005**, *2*, 81.
[76] Bresnahan, W.A.; Boldogh, I.; Chi, P.; Thompson, E.A.; Albrecht, T. *Virology*, **1997**, *231*, 239.
[77] Schang, L.M.; Phillips, J.; Schaffer, P.A. *J. Virol*., **1998**, *72*, 5626.
[78] Redfield, D.C.; Richman, D.D.; Oxman, M.N.; Kronenberg, L.H. *Infect. Immun*., **1981**, *32*, 1216.

[79] Durantel, D.; Branza-Nichita, N.; Carrouée-Durantel, S.; Butters, T.D.; Dwek, R.A.; Zitzmann, N. *J. Virol.*, **2001**, *75*, 8987.

[80] Marles, R.J.; Hudson, J.B.; Graham, E.A.; Soucy-Breau, C.; Morand, P.; Compadre, R.L.; Compadre, C.M.; Towers, G.H.; Arnason, J.T. *Photochem. Photobiol.*, **1992**, *56*, 479.

[81] Stanberry, L.R.; Bourne, N.; Bravo, F.J; Bernstein, D.I. *J. Med.Virol.*, **1992**, *38*, 142.

[82] Si, X.; Wang, Y.; Wong, J.; Zhang, J.; McManus, B.M.; Luo, H. *J. Virol.*, **2007**, *81*, 3142.

[83] Dikici, I.; Mehmetoglu, I.; Dikici, N.; Bitirgen, M.; Kurban, S. *Clin. Biochem.*, **2005**, *38*, 1141.

[84] Joint United Nations Programme on HIV/AIDS, "Overview of the global AIDS epidemic", **2006** Report on the global AIDS epidemic (b) Paris, A.; Strukelj, B.; Renko, M.; Turk, V.; Pukl, M.; Umek, A.; Korant, B.D. *J. Nat. Prod.*, **1993**, *56*, 1426.

[85] Chang, C.T.; Doong, S.L.; Tsai, I.L.; Chen, I.S. *Phytochemistry*, **1997**, *45*, 1419.

[86] Fortin, H.; Tomasi, S.; Jaccard, P.; Robin, V.; Boustie, J. *Chem. Pharm. Bull.*, **2001**, *49*, 619.

[87] Sakagami, H.; Asano, K.; Satoh, K.; Takahashi, K.; Kobayashi, M.; Koga, N.; Takahashi, H.; Tachikawa, R.; Tashiro, T.; Hasegawa, A.; Kurihara, K.; Ikarashi, T.; Kanamoto, T.; Terakubo, S.; Nakashima, H.; Watanabe, S.; Nakamura, W. *In Vivo*, **2007**, *21*, 499.

[88] Hsieh, P.-W.; Chang, F.-R.; Lee, K.-H.; Hwang, T.-L.; Chang, S.M.; Wu, Y.C. *J. Nat. Prod.*, **2004**, *67*, 1175.

[89] Lee, J.S.; Kim, H.J; Lee, Y.S. *Planta Med.*, **2003**, *69*, 859.

[90] Olivero-Verbel, J.; Pacheco-Londono, L. *J. Chem. Inform. Comput. Sci.*, **2002**, *42*, 1241.

[91] Nishibe, S.; Ono, K.; Nakane, H.; Kawamura, T.; Noro, Y.; Tanaka, T. *Nat. Med.*, **1997**, *51*, 547

[92] Kim, H.J; Woo, E.-R.; Shin, C.-G.; Park, H. *J. Nat. Prod.*, **1998**, *61*, 145.

[93] Critchfield, J.W.; Butera, S.T.; Folks, T.M. *Retroviruses*, **1996**, *12*, 39.

[94] Mahmood, N.; Pizza, C.; Aquino, R.; De Tommasi, N.; Piacente, S.; Colman, S.; Burke, A.; Hay, A.J. *Antiviral Res.*, **1993**, *22*, 189

[95] Schinazi, R.F.; Chu, C.K.; Babu, J.R.B.; Oswald, V.; Saalman, D.L.; Erickson, B.F. *Antiviral Res.,* **1990**, *13*, 265.

[96] Robin, V.; Boustie, J.; Amoros, M.; Girre, L. *Pharm. Pharmacol. Commun.*, **1998**, *4*, 61.

[97] Tuli, V. *Antimicrob. Agents Chemother.* **1974,** *5(5),* 485.

[98] Lyu, S.-Y.; Rhim, J.Y.; Park, W.B. *Arch. Pharm. Res.*, **2005**, *28*, 1293

[99] Ma, W.; Ma, X.; Huang, H.; Zhou, P.; Chen, D. *Chem. Biodivers.*, **2007**, *4*, 966.

[100] Chattopadhyay D.; In *Ethnomedicinal Antivirals: Scope and Opportunity* Ed.; Ahmad,I.; Aqil, F.; Owais, M. Wiley-VCH Verlag GmbH & Co, **2007**

[101] Benencia, F.; Courrèges, M.C. *Phytother. Res.*, **2000,** *14,* 495.

[102] Li, H.; Zhou, C.X.; Pan, Y.; Gao, X.; Wu, X.; Bai, H.; Zhou, L.; Chen, Z.; Zhang, S.; Shi, S.; Luo, J.; Xu, J.; Chen, L.; Zheng, X.; Zhao, Y. *Planta Med.*, **2005**, *71*, 1128.

[103] Likhitwitayawuid, K.; Supudompol, B.; Sritularak, B.; Lipipun, V.; Rapp, K.; Schinazi, R. F. *Pharmaceutical Biology* (Philadelphia, PA, United States), **2005**, *43*, 651.

[104] El Sohly, H.N.; El-Feraly, F.S.; Joshi, A.S.; Walker, L.A. *Planta Med.*, **1997**, *63*, 384.

[105] Lin, L.-C.; Kuo, Y.-C.; Chou, C.-J. *Planta Med.*, **2000**, *66*, 333.

[106] Du, J.; He, Z.-D.; Jiang, R.-W.; Ye, W.-C.; Xu, H.-X.; But, P.P.H. *Phytochemistry (Elsevier)*, **2003**, *62*, 1235.

[107] Jou, S.-J.; Chen, C.-H.; Guh, J.-H.; Lee, C.-N.; Lee, S.S. *J. Chin. Chem. Soc. (Taipei, Taiwan)*, **2004**, *51*, 827.

[108] Peterson, M.; Simmonds, M.S. *Phytochemistry*, **2003,** *62,* 121.

[109] Lin, L.C.; Kuo, Y.-C.; Chou, C.J. *Planta Med.*, **2000**, *66*, 333.

[110] Afifi, M.S.; Salama, O.M.; Hassan, M.A.; Naeim, Z.-E.M.; Maatooq, G.T.; Mohamed, K.F. *J. Pharm. Sci.*, **2001**, *17*, 25.

[111] Bunyapraphatsara, N.; Dechsree, S.; Yoosook, C.; Herunsalee, A.; Panpisutchai, Y. *Phytomedicine*, **2000**, *6*, 421.

[112] Okamoto, T.; Yoshida, S.; Kobayashi, T.; Okabe, S. *Int. J. Mol. Med.*, **2001**, *7*, 177. (b) Oh, H.; Lee, H.S.; Kim, T.; Chai, K.Y.; Chung, H.T.; Kwon, T.O.; Jun, J.Y.; Jeong, O.S.; Kim, Y.C.; Yun, Y.G. *Planta Med.,* **2002**, *68*, 463.

[113] Li, H.; Zhou, C.X.; Pan, Y.; Gao, X.; Wu, X.; Bai, H.; Zhou, L.; Chen, Z.; Zhang, S.; Shi, S.; Luo, J.; Xu, J.; Chen, L.; Zheng, X.; Zhao, Y. *Planta Med.*, **2005**, *71*, 1128.

[114] Shin, M.S.; Kang, E.H.; Lee, Y.I. *Antiviral Res.*, **2005**, *67*, 163.

[115] Cai, S.-Q.; Wang, R.; Yang, X.; Shang, M.; Ma, C.; Shoyama, Y. *Chem. Biodiver.*, **2006**, *3*, 343

[116] Nagai, T.; Suzuki, Y.; Tomimori, T.; Yamada, H. *Bio. Pharm. Bull.*, **1995**, *18*, 295.

[117] Sidwell, R.W.; Huffman, J.H.; Moscon, B.J.; Warren, R.P. *Chemotherapy (Basel, Switzerland),* **1994**, *40*, 42.

[118] Quaglia, M.G.; Desideri, N.; Bossu', E.; Sgro, R.; Conti, C. *Chirality,* **1999,** *11,* 495. (b) Douglas, R.M.; Chalker, E.B.; Treacy, B. *Cochrane Database Syst. Rev.,* **2000**, *2*, CD000980. (c) Melchart, D.; Linde, K.; Fischer, P.; Kaesmayr, *J. Cochrane Database Syst. Rev.*, **2000**, *2*, CD000530.

[119] Desideri, N.; Conti, C.; Mastromarino, P.; Mastropaolo, F. *Antivir. Chem. Chemother.*, **2000**, *11*, 373 (b) Weber, N.D.; Andersen, D.O.; North, J.A.; Murray, B.K.; Lawson, L.D.; Hughes, B.G. *Planta Med.*, **1992**, *58*, 417.

[120] Tait, S.; Salvati, A. L.; Desideri, N.; Fiore, L. *Antiviral Res.*, **2006**, *72*, 252. (b) Singh, S.; Malik, B.K.; Sharma, D.K. *Chem. Biol. Drug Des.*, **2007**, *69*, 191.

[121] Amaral, A.C.F.; Kuster, R.M.; Goncalves, J.L.S.; Wigg, M.D. *Fitoterapia*, **1999**, *70*, 293.

[122] Desideri, N.; Oliveri, S.; Stein, M.L.; Sgro, R.; Orsi, N.; Conti, C. *Antivir. Chem. Chemother.*, **1997**, *8*, 545.

[123] Chiang, L.C.; Chiang, W.; Liu, M.C.; Lin, C.C. *J. Antimicrob. Chemother.*, **2003**, *52*, 194.

[124] Moriarty, R. M.; Naithani, R.; Hirtopeanu, A.; Gao, K.; Kazerani, S.; Badlani, R.; Staszewski, J. P.; Zitzmann, N.; Dwek, R. A.; Jacob, G. S.; Picker, D.; Mehta, A.; Block, T. M. N. Alkyl deoxynojirimycins as antiviral compounds. *Abstracts of Papers*, 224th ACS National Meeting, Boston, MA, United States, August 18-22, **2002** (b) Mehta, A.; Ouzounov, S.; Jordan, R.; Simsek, E.; Lu, X.; Moriarty, R.M.; Jacob, G.; Dwek, R.A.; Block, T.M. *Antivir. Chem. Chemother.*, **2002**, *13,* 299.

[125] De Almeida, A.P.; Miranda, M.M.F.S.; Simoni, I.C.; Wigg, M.D.; Lagrota, M. H.C.; Costa, S.S. *Phytother. Res.*, **1998**, *12*, 562.

[126] Docherty, J.J.; Sweet, T.J.; Bailey, E.; Faith, S.A.; Booth, T. *Antiviral Res.*, **2006**, *72*, 171.

[127] Kiat, T.S.; Pippen, R.; Yusof, R.; Ibrahim, H.; Khalid, N.; Rahman, N.A. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 3337.

[128] Bae, E.-A.; Han, M.J.; Lee, M.; Kim, D.H. *Biol. Pharm.Bull.*, **2000**, *23*, 1122.

[129] Li, B.Q.; Fu, T.; Gong, W.H.; Dunlop, N.; Kung, H. F.; Yan, Y.; Kang, J.; Wang, J. M. *Immunopharmacology*, **2000**, *49*, 295.

[130] Rusak, G.; Krajacic, M.; Plese, N. *Antiviral Res.*, **1997**, *36*, 125.

[131] Mitrocosta, D.; Mitaku, S.; Axarlis, S.; Harvala, C.; Malamas, M. *Planta Med.*, **2000**, *66*, 377.

[132] Gerencer, M.; Turecek, P.L.; Kistner, O.; Mitterer, A.; Savidis-Dacho, H.; Barrett, N.P. *Antiviral Res.,* **2006**, *72,* 153.

[133] Sanchez, I.; Gomez-Garibay, F.; Taboada, J.; Ruiz, B.H. *Phytother. Res.*, **2000**, *14*, 89.

[134] Thyagarajan, S.P.; Subramanian, S.; Thirunalasundari, T.; Venkateswaran, P.S.; Blumberg, B.S. *Lancet*, **1988**, *2*, 764.

[135] Thyagarajan, S.P.; Jayaram, S.; Valliammai, T.; Madanagopalan, N.; Pal, V.G.; Jayaraman, K. *Lancet*, **1990**, *336*, 949.

[136] Thamlikitkul, V.; Wasuwat, S.; Kanchanapee, P. *J. Med. Assoc. Thailand,* **1991**, *74*, *381*.

[137] Berk, L.; de Man, R.A.; Schalm, S.W.; Labadie, R.P.; Heitjtink, R.A. *J. Hepatol.*, **1991**, *12*, 405.

[138] Zhang, J.L.; He, W.N.; Ye, P. *Chin. J. Integr. Trad. Western Med. Liver Dis.*, **1992**, *2*, 8.

[139] Milne, A.; Hopkirk, N.; Lucas, C.R.; Waldon, J.; Foo, Y. *N Z Med. J.*, **1994**, *107*, 253.

[140] Peng, X.C.; Liu, X.L.; Liu, S.C.; Yang, D.Z.; Wang, W.J.; Zhang, H.J. *Chinese J. Experi. Clin. Virol.*, **1993**, 7, 306.

[141] Huang, R.L. Huang, Y.L.; Ou, J.C.; Chen, C.C.; Hsu, F.L.; Chang, C. *Phytother. Res.*, **2003**, *17*, 449.

[142] Zhu, F.M.; Zhang, J.Q.; Zhang, X.Z.; Zhang X.M. *Chin. J. Integr. Trad. Western Med. Liver Dis.*, **1992**, *2*, 10.

[143] Cao, W.Z.; Liu, J.Q.; Cao, D.Y, Su, F.; Xu, S.G. *J. Chin. Materia Med.*, **1998**, *23*, 180.

[144] Huang, Z.R.; Zhong, J.P.; Zhu, G.L.; Chen, Y.R.; Wang, G.Q. *Chin. J. Clin. Hepatol.* **1993**, *9*, 108.

[145] Wang, M.; Cheng, H.; Li, Y.; Meng, L.; Zhao, G.; Mai, K. *J. Lab. Clin. Med.*, **1995**, *126,* 350.
[146] Su, X.; Chen, H.; Wang, L.; Jiang, C.; Liu, J.; Zhao, M.; Ma, X.; Zhao, Y.; Han, D. *J. Trad. Chin. Med.*, **1984**, *4*, 127.
[147] Iino, S.; Tango, T.; Matsushima, T.; Toda, G.; Miyake, K.; Hino, K.; Kumada, H.; Yasuda, K.; Kuroki, T.; Hirayama, C.; Suzuki, H. *Hepatol. Res.*, **2001**, *19*, 31.
[148] Doshi, J.C.; Vaidya, A.B. Antarkar, D.S.; Deolalikar, R.; Antani, D.H. *Indian J. Gastroenterol.,* **1994**, *13*, 7.
[149] Tusenius, K.J.; Spoek, J.M.; Kramers, C.W. *Complement Ther. Med.*, **2001**, *9*, 12.
[150] van Rossum, T.G.J.; Vulto, A.G.; Hop, W.C.J.; Brouwer, J.T.; Niesters, H.G.M.; Schalm, S.W. *J. Gastroenterol. Hepatol.*, **1999**, *14*, 1093.
[151] Tsubota, A.; Kumada, H.; Arase, Y.; Chayama, K.; Saitoh, S.; Ikeda, K.; Kobayashi, M.; Suzuki, Y.; Murashima, N. *Eur. J. Gastroenterol. Hepatol.*, **2002**, *11*, 1077.
[152] van Rossum, T.G.J.; Vulto, A.G.; Hop, W.C.J.; Schalm, S.W. *Am. J. Gastroenterol.*, **2001**, *96*, 2432.
[153] Mehrotra, R.; Rawat, S.; Kulshreshtha, D.K. *Indian J. Med. Res.*, **1990**, *92*, 133.
[154] Stauder, G.; Kabil, S. *J. Immunother.*, **1997**, *13*, 153.
[155] Suzuki, H.; Ohta, Y.; Tekino, T.; Fujisawa, K.; Hirayama C. *Asian Med. J.*, **1983**, *26*, 423.
[156] Miyake, K.; Tango, T.; Ota, Y.; Mitamura, K.; Yoshiba, M.; Kako, M.; Hayashi, S.; Ikeda, Y.; Hayashida, N.; Iwabuchi, S.; Sato, Y.; Tomi, T.; Funaki, N.; Hashimoto, N.; Umeda, T.; Miyazaki, J.; Tanaka, K.; Endo, Y.; Suzuki, H. *J.Gastroenterol. Hepatol.*, **2002**, *17*, 1198.
[157] Vailaii, A.; Arista, I.; Sozze, E. *Fitoterapia*, **1993**, *64*, 219.
[158] Narendranathan, M.; Remla, A.; Mini, P.C.; Satheesh, P. *Trop. Gastroenterol.*, **1999**, *20*, 164.
[159] Parés, A.; Planas, R.; Torres, M.; Caballería, J.; Viver, J.M.; Acero, D.; Panés, J.; Rigau, J.; Santos, J.; Rodés, J. *J. Hepatol*., **1998**, *28*, 731.
[160] Ferenci, P.; Dragosics, B.; Dittrich, H.; Frank, H.; Benda, L.; Lochs, H.; Meryn, S.; Base, W.; Schneider, B. *J. Hepatol.*, **1989**, *9*, 105.
[161] Thom, E.; Zakay-Rones, Z.; Wollan, T.; Wadstein, J. *Proc.of the 15th International Conference on Antivir. Res*., Prague, Czech Republic, March **2002**.
[162] Zakay-Rones, Z.; Varsano, N.; Zlotnik, M.; Manor, O.; Regev, L.; Schlesinger, M.; Mumcuoglu, M. *J. Alter. Complementary Med.*, **1995**, *1*, 361.
[163] Melchoir, J.; Palm, S.; Wikman, G. *Phytomedicine*, **1996**, *3*, 315.

Received: 30 November, 2007 Revised: 23 January, 2008 Accepted: 23 January, 2008

[164] Caceres, D.D.; Hancke, J.L.; Burgos, R.A.; Sandberg, F.; Wikman, G.K. *Phytomedicine*, **1999**, *6*, 217.
[165] Hancke, J.; Burgos, R.; Caceres, D.; Wikman, G. *Phyto., Res.*, **1995**, *9*, 559.
[166] Josling, P. *Adv. Ther.*, **2001**, *18*, 189.
[167] Phillpotts, R.J; Wallace, J.; Tyrrell, D.A.; Freestone, D.S.; Shepherd, W.M. *Arch.Virol.*, **1983**, *75*, 115.
[168] Tanamly, M.D.; Tadros, F.; Labeeb, S. *Dig. Liver Dis*., **2004**, *36*, 752.
[169] Gordon, A.; Hobbs, D.A.; Bowden, D.S. *J. Gastroenterol. Hepatol*., **2006**, *21*, 275.
[170] Melhem, A.; Stern, M.; Shibolet, O.; Israeli, E.; Ackerman, Z.; Pappo, O.; Hemed, N.; Rowe, M.; Ohana, H.; Zabrecky, G.; Cohen, R.; Ilan, Y. *J. Clin. Gastroenterol.*, **2005**, *39*, 737.
[171] Strickland, G.T.; Tanamly, M.D.; Tadros, F. *Dig. Liver Dis*., **2005**, *37*, 542.
[172] El-Zayadi, A.-R.; Attia, M.; Badran, H.M. *Liver Int*., **2005**, *25*, 746.
[173] Koytchev, R.; Alken, R.G.; Dundarov, S. *Phytomedicine*, **1999**, *6*, 225.
[174] Wobling, R.H.; Leonhardt, K. *Phytomedicine,* 1**994**, *1*, 25
[175] Syed, T.A.; Cheema, K.M.; Ashfaq, A.S.; Holt, A.H. Jr. *J. Eur. Acad. Derm. Venerol.*, **1996**, *7*, 294.
[176] Syed, T.A.; Afzal, M.; Ashfaq, A.S.; Holt, A.H.; Ali, A.S.; Ahmad, S.H. *J. Dermatol. Treat.*, **1997**, *8*, 99.
[177] Jayavasu, C.; Balachandra, K.; Sangkitporn, S.; Maharungraungrat, A.; Thavatsupa, P.; Bunjob, M.; Chavalittumrong, P.; Dechatiwongse, N.; Ayudhaya, T.; Sittisomwong, N. *Commun. Dis. J.*, **1992**, *18*, 152.
[178] Carson, C.F.; Ashton, L.; Dry, L.; Smith, D.W.; Riley, T.V. *J. Antimicrob. Chem.,* **2001**, *48*, 450.
[179] Saller, R.; Buechi, S.; Meyrat, R.; Schmidhauser, C. *Forsch Komplementarmed Klass Naturheilkd*, **2001**, *8*, 373.
[180] Sangkitporn, S.; Chaiwat, S.; Balachandra, K.; Na-Ayudhaya, T.D.; Bunjob, M.; Jayavasu, C. *J. Med. Assoc. Thailand*, **1995**, *178*, 624.
[181] Charuwichitratana, S.; Wongrattanapasson, N.; Timpatanapong, P.; Bunjob, M. *Int. J. Dermat.*, **1996**, *35*, 665.
[182] Calabrese, C.; Berman, S.H.; Babish, J.G.; Ma, X.; Shinto, L.; Dorr, M.; Wells, K.; Wenner, C.A.; Standish, L.J. *Phytother., Res.,* **2000**, *14,* 333.
[183] Durant, J.; Chantre, G.; Gonzalez, G.; Vandermander, J.; Halfon, P.; Rousse, B.; Guedon, D.; Rahelinirina, V.; Chamaret, S.; Montagnier, L.; Dellamonica, *Phytomedicine*, **1998**, *5*, 1.
[184] Gotoh, Y.; Tada, K.; Yamada, K.; Minamitani, M.; Negishi, M.; Fujimaki, M.; Ikematsu, S.; Hada, M.; Mori, K.; Ito, M.; Shigeta, S.; Nakashima, H.; Yamamoto, N.; Shiokawa, Y. *Igaku Ayumi*, **1987**, *140*, 619.