A REVIEW

Novel antiviral agents: a medicinal plant perspective

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1. SUMMARY

Several hundred plant and herb species that have potential as novel antiviral agents have been studied, with surprisingly little overlap. A wide variety of active phytochemicals, including the flavonoids, terpenoids, lignans, sulphides, polyphenolics, coumarins, saponins, furyl compounds, alkaloids, polyines, thiophenes, proteins and peptides have been identified. Some volatile essential oils of commonly used culinary herbs, spices and herbal teas have also exhibited a high level of antiviral activity. However, given the few classes of compounds investigated, most of the pharmacopoeia of compounds in medicinal plants with antiviral activity is still not known. Several of these phytochemicals have complementary and overlapping mechanisms of action, including antiviral effects by either inhibiting the formation of viral DNA or RNA or inhibiting the activity of viral reproduction. Assay methods to determine antiviral activity include multiple-arm trials, randomized crossover studies, and more compromised designs such as nonrandomized crossovers and pre- and post-treatment analyses. Methods are needed to link antiviral efficacy/potency- and laboratory-based research. Nevertheless, the relative success achieved recently using medicinal plant/herb extracts of various species that are capable of acting therapeutically in various viral infections has raised optimism about the future of phyto-antiviral agents. As this review illustrates, there are innumerable potentially useful medicinal plants and herbs waiting to be evaluated and exploited for therapeutic applications against genetically and functionally diverse viruses families such as *Retroviridae*, *Hepadnaviridae* and *Herpesviridae*.

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2. INTRODUCTION

Viruses are obligate intracellular parasites, which contain little more than bundles of gene strands of either RNA or DNA, and may be surrounded by a lipid-containing envelope (Wagner and Hewlett 1999). Yet viruses are far from simple. Unlike bacterial cells, which are free-living entities, viruses utilize the host cell environment to propagate new viruses. They use the reproductive machinery of cells they invade causing ailments as benign as a common wart, as irritating as a cold, or as deadly as what is known as the bloody African fever. The viruses that cause Lassa fever and Ebola fever and the retrovirus that causes acquired immunodeficiency syndrome (AIDS) are examples that researchers call hot agents – viruses that spread easily, kill sometimes swiftly, and for which there is no cure or vaccine (Peter 1994).

Viruses have numerous invasion strategies. Each strain of virus has its own unique configuration of surface molecules (Wagner and Hewlett 1999). These surface molecules work like keys in a lock, enabling viruses to enter into hosts by precisely fitting the molecules on their surfaces to those on

the membranes of target cells. The success of viruses in evolution has been assured by four general attributes: genetic variation, variety in means of transmission, efficient replication within host cells, and the ability to persist in the host (Wagner and Hewlett 1999). As a consequence viruses have adapted to all forms of life and have occupied numerous 'ecological niches' resulting in widespread diseases in humans, livestock and plants.

2.1 Viral infection control

Control of viral infections, like any other kind of infection control, can be affected either as a prophylactic (protective) measure or therapeutically, in order to control and alleviate a viral infection, which has already been established in the host. Unlike bacterial, fungal and parasitic infections, viruses are not autonomous organisms and therefore, require living cells in which to replicate. Consequently, most of the steps in their replication involve normal cellular metabolic pathways, and this makes it difficult to design a treatment to attack the virion directly, or its replication, without accompanying adverse effects on the infected cells (Wagner and Hewlett 1999). Fortunately, we now know that many viruses have unique features in their structure or in their replication cycles, and these constitute potential targets. In fact, successful antiviral chemotherapy has been achieved against the herpes virus with the development of acycloguanosine, sold as 'acyclovir', because it interferes with certain key viral enzymes that have distinctive affinities for different nucleotide analogues (Wagner and Hewlett 1999). Viral enzymes play a key role in triggering disease. If viral enzymes could be neutralized, viral replication would not take place. The proteolytic processing of viral polyprotein precursors by a viral proteinase is essential for maturation of the virus. Designing specific inhibitors for each of viral protease is thus a desirable objective.

2.2 Overview of medicinal plants worldwide

There is currently a large and ever-expanding global population base that prefers the use of natural products in treating and preventing medical problems. This has influenced many pharmaceutical companies to produce new antimicrobial formulations extracted from plants or herbs.

At present, plant and herb resources are unlimited, as far as the search for useful phyto-chemicals is concerned; but these resources are dwindling fast, due to the onward march of civilization. We have barely scraped the surface in our efforts to exploit the plant world for antimicrobials (namely, antiviral, antibacterial and antifungal compounds). Although a significant number of studies have used known purified plant chemicals, very few screening programmes have been initiated on crude plant materials.

Virtually all cultures around the globe have relied historically, and continue to rely on medicinal plants for primary health care. There is currently a worldwide upsurge in the use of herbal preparations and the active ingredients isolated from medicinal plants in health care. Natural products from plants traditionally have provided the pharmaceutical industry with one of its most important sources of 'lead' compounds and up to 40% of modern drugs are derived from natural sources, using either the natural substance or a synthesized version.

3. ANTIVIRAL ACTIVITY OF HERBAL MEDICINE AGAINST SELECTED VIRUSES

Many traditional medicinal plants have been reported to have strong antiviral activity and some of them have already been used to treat animals and people who suffer from viral infection (Hudson 1990; Venkateswaran et al. 1987; Thyagarajan et al. 1988, 1990). Research interests for antiviral agent development was started after the Second World War in Europe and in 1952 the Boots drug company at Nottingham, England, examined the action of 288 plants against influenza A virus in embryonated eggs. They found that 12 of them suppressed virus amplification (Chantrill et al. 1952). During the last 25 years, there have been numerous broad-based screening programmes initiated in different parts of the globe to evaluate the antiviral activity of medicinal plants for in vitro and in vivo assays. Canadian researchers in the 1970s reported antiviral activities against herpes simplex virus (HSV), poliovirus type 1, coxsackievirus B5 and echovirus 7 from grape, apple, strawberry and other fruit juices (Konowalchuk and Speirs 1976a,b, 1978a,b).

One hundred British Columbian medicinal plants were screened for antiviral activity against seven viruses (McCutcheon et al. 1995). Twelve extracts were found to have antiviral activity at the concentrations tested. The extracts of *Rosa nutkana* and *Amelanchier alnifolia* were very active against an enteric corona virus. A root extract of *Potentilla arguta* and a branch tip extract of *Sambucus racemosa* completely inhibited respiratory syncytial virus (RSV). An extract of *Ipomopsis aggregata* demonstrated good activity against parainfluenza virus type 3. A *Lomatium dissectum* root extract completely inhibited the cytopathic effects of rotavirus. In addition to these, extracts prepared from *Cardamine angulata*, *Conocephalum conicum*, *Lysichiton americanum*, *Polypodium glycyrrhiza* and *Verbascum thapsus* exhibited antiviral activity against herpes virus type 1.

The extracts of 40 different plant species have been used in traditional medicine and were investigated for antiviral activity against a DNA virus, human cytomegalovirus (HCMV), and two RNA viruses, Ross River virus (RRV) and poliovirus type 1, at noncytotoxic concentrations (Semple et al. 1998). The most active extracts were the

aerial parts of *Pterocaulon sphacelatum* (Asteraceae) and roots of *Dianella longifolia* var. grandis (Liliaceae), which inhibited poliovirus type 1 at concentration of 52 and 250 μg ml⁻¹, respectively. The same authors concluded that the extracts of *Euphorbia australis* (Euphorbiaceae) and *Scaevola spinescens* (Goodeniaceae) were the most active against HCMV whilst, extracts of *Eremophila latrobei* subsp. *glabra* (Myoporaceae) and *Pittosporum phylliraeoides* var. *microcarpa* (Pittosporaceae) exhibited antiviral activity against RRV.

The human rotavirus (HRV), RSV and influenza A virus were susceptible to a liquid extract from *Eleutherococcus senticosus* roots. In contrast, the DNA viruses, adenovirus and HSV type 1 virus (HSV-1) were not inhibited by the same plant extract (Glatthaar-Saalmuller *et al.* 2001). They concluded that the antiviral activity of *Eleutherococcus senticosus* extract is viral RNA dependant.

Related studies also showed that influenza RNA was inhibited by a water-soluble extract of *Sanicula europaea* (L.) (Turan *et al.* 1996). In a later study of Karagoz *et al.* (1999) it was shown that an acidic fraction obtained from the crude extract of *Sanicula europaea* was the most active fraction in inhibiting human parainfluenza virus type 2 replication at noncytotoxic concentrations. By comparison, ethanol extraction abolished the antiviral activity. The plausible explanation is that the antiviral activity could 'disappear' during the course of fractionation.

Another example, *Myrcianthes cisplatensis* showed *in vitro* anti-RSV but not anti-HSV-1 or anti-adenovirus serotype 7 (DNA virus) (Kott *et al.* 1999). In contrast, other medicinal plants, for example *Nepeta coerulea*, *Nepeta nepetella*, *Nepeta tuberosa*, *Sanguisorba minor magnolii* and *Dittrichia viscose* showed clear antiviral activity against DNA and RNA viruses, i.e. HSV-1 and VSV in addition to poliovirus type 1 in the case of *Dittrichia viscose* (Abad *et al.* 2000). The *Azadirachta indica* leaf extract was found to be active against a number of viruses such as smallpox (DNA), chicken pox (DNA), poxvirus (DNA), poliomyelitis (RNA) and herpes viruses (DNA) (Rao *et al.* 1969; Kaii-a-Kamb *et al.* 1992). An extract of the cactus plant *Opuntia streptacantha* inhibited intracellular DNA and RNA virus replication and inactivated extracellular virus, such as HSV, equine herpes virus, pseudorabies virus and influenza virus (Ahmad *et al.* 1996). The *Bergenia ligulata*, *Nerium indicum* and *Holoptelia integrifolia* plants exhibited considerable antiviral activities against influenza virus (RNA) and HSV (DNA) (Rajbhandari *et al.* 2001).

4. MARINE HERBS AND ANTIVIRAL ACTIVITIES

Natural product research is increasingly turning to marine herbs as a source of natural products and is currently in preclinical and clinical evaluation. Others show promising biological activities in *in vitro* and *in vivo* assays (Konig and Wright 1996; Blunden 2001). The antiviral properties of marine algae have been addressed (Chamorro *et al.* 1996; Siddhanta *et al.* 1997; Berge *et al.* 1999; Nicoletti *et al.* 1999). Preclinical testing suggests that *Spirulina*, a unicellular filamentous cyanobacteria (formerly called 'blue-green algae'), has several therapeutic attributes such as cholesterol regulation, immunological, antiviral and antimutagenic properties (Chamorro *et al.* 1996). Strain-specific anti-influenza virus inhibitory activity, based on the reproduction of influenza viruses in tissue cultures, was reported for marine algae of the Bulgarian Black Sea coast (Serkedjieva *et al.* 2000). Water extracts from *Haslea ostrearia* and the red marine alga *Polysiphonia denudata* from the Bulgarian Black Sea coast, respectively, inhibited the reproduction of HSV in cell cultures and affected adsorption and the intracellular stages of viral replication as demonstrated by the reduction of virus-induced cytopathic effect and viral infectivity (Berge *et al.* 1999; Serkedjieva 2000a). In addition, the water-soluble fraction of *Haslea ostrearia* has delayed HIV-1-induced syncitia formation on MT4 cells (Berge *et al.* 1999).

The inhibitory effect of marine algae was investigated and found that cyanovirin-N, an 11 kDa protein from blue-green alga irreversibly inactivated HIV and also aborted cell-to-cell fusion and transmission of HIV, due to its high-affinity interaction with gp120 (De Clercq 2000). The presence of various sulphated polysaccharide groups extracted from seaweeds and alga have exhibited many biological properties, for example anti-HIV and anti-HSV activities and also the inhibition of viral adsorption processes (De Clercq 2000; Schaeffer and Krylov 2000; Duarte *et al.* 2001). It is well known that the presence of the sulphate group is necessary for antiviral activity, and potency increases with the degree of sulphation (Schaeffer and Krylov 2000). Given the few classes of compounds investigated thus far, most of the antiretroviral activity in algae is unknown.

5. THE COMMON CLASS OF ANTIVIRAL COMPOUNDS PRESENT IN MEDICINAL PLANTS

The development of viral resistance towards antiviral agents enhances the need for new effective compounds against viral infections. Medicinal plants have a variety of chemical constituents, which have the ability to inhibit the replication cycle of various types of DNA or RNA viruses. Compounds from natural sources are of interest as possible sources to control viral infection. In this context various research groups in Asia, Far East, Europe and America have given particular attention to develop antiviral agents from their

native traditional plant medicines. Some typical examples of such medicines and their antiviral activities are shown in Table 1.

The antimicrobial activities of plant oils and extracts have been recognized for many years. Recently, the oil of *Melaleuca alternifolia* (tea tree) has gained widespread acceptance and it is now the principal antimicrobial preservative in a range of pharmaceutical cosmetics for external use, such as face and hand washes, pimple gels, vaginal creams, foot powders, shampoos, conditioners and veterinary skin care products (Cox *et al.* 2001). The antiviral action of essential oils of *Melaleuca alternifolia* and eucalyptus oil exhibited a high level of antiviral activity against HSV-1 and HSV-2 in viral suspension tests (Schnitzler *et al.* 2001). The activities of anti-herpes components could be the result of terpinen-4-ol (Cox *et al.* 2001). Italian medicinal plants and food medicines were reviewed (Pieroni 2000) and it was found that essential oil obtained from *Santolina insularis* had direct antiviral effects on both HSV-1 and HSV-2 and also inhibited cell-to-cell transmission of both herpes types (De Logu *et al.* 2000). Sandalwood oil, the essential oil of *Santalum album* (L.), showed a dose-dependent effect against HSV-1 but not HSV-2 with no reported cytotoxicity (Benencia and Courreges 1999). Recently the antiviral effect of black seed oil (BSO) from *Nigella sativa* was investigated using murine cytomegalovirus (MCMV) as a model (Salem and Hossain 2000). Their results show that BSO exhibited a striking antiviral effect against MCMV infection, which may be mediated by increasing one's innate immunity.

Polyphenols and the proanthocyanidins extracted from *Hamamelis virginiana* bark and two new hydrolysable tannins, shephagenins A and B, isolated along with hippophaenin A and strictinin from the leaf extract of *Shepherdia argentea*, showed a remarkable inhibitory activity against HSV-1 (Erdelmeier *et al.* 1996) and HIV-1 reverse transcriptase (RT) (Yoshida *et al.* 1996). The inhibitory effect of the *Shepherdia argentea* leaf extract on HIV-1 RT was found to be caused by tannins, and their activities were stronger than that of (−)epigallocatechin gallate as a positive control (Yoshida *et al.* 1996).

In an early study of plant viral infection, Cadman (1960) suggested that polyphenolic extracts of the leaf of *Rubus idaeus* (raspberry) probably act against most viruses by clumping the virus particles together into complexes, which are largely noninfective. Hudson (1990) deduced that viral inactivation *in vitro* is directly attributable to preferential binding of the polyphenol to the protein coat of the virus, whereas, in a systematic study of the antiviral activity of a very wide range of natural products Van den Berghe *et al.* (1986) concluded that polyphenols act principally by binding to the virus and/or the protein of the host cell membrane and thus arrest absorption of the

virus. Sakagami *et al.* (1995) have put forward a number of possible mechanisms whereby polyphenols may exert their antiviral action. They suggested that the major part of the antiviral activity in polyphenols probably derives from their direct inactivation of the virus and/or from inhibition of the virus binding to the cells. They also noted that although polyphenols are known to inhibit viral replication enzymes (such as RT for HIV and RNA polymerase for influenza virus) and other enzymes (e.g. poly(ADP-ribose) glycohydrolase), these effects seem to be rather nonspecific. The most pronounced *in vitro* selectivity of anti-influenza and anti-herpes type 1 and type 2 action were confirmed against polyphenolic complexes isolated from the Bulgarian medicinal plant *Geranium sanguineum* (L.) (Serkedjieva and Hay 1998; Serkedjieva and Ivancheva 1999). Although polyphenols were shown to have a broad antiviral spectrum *in vitro*, their corresponding properties *in vivo* have not been well established (Sakagami *et al.* 1995).

A peptide isolated from the leaves of the Argentinean plant *Melia azedarach* has a molecular weight of 5000–6000 (Table 1), which may be common in many plants (Hudson 1990). The peptide was evaluated with mice inoculated with HSV-1 strain (Alche *et al.* 2000). Infected animals treated or not with meliacine were observed carefully for the development of stromal keratitis and the clinical scoring was followed 14 days postinfection. It was found that meliacine exerted a strong antiviral action on HSV-1 induced ocular disease in mice with no evidence of toxic effects. There have also been reports of the beneficial effects of meliacine in helping to control the Junin hemorrhagic fever virus by inhibiting the multiplication of Junin virus in vero cells treated with the compound before infection or immediately after virus adsorption (Castilla *et al.* 1998). It also inhibited the multiplication of foot and mouth disease virus in BHK-21 cells (Wachsman *et al.* 1998). Analysis of early events following infection demonstrated that meliacine blocks virus penetration by preventing the uncoating step, but the addition of meliacine at different times after infection indicated that meliacine also interferes with the release of infectious particles and inhibits the low-pH-induced fusion of infected cells (Castilla *et al.* 1998; Wachsman *et al.* 1998). Taken together, these results suggest that meliacine affects two events of the virus replicative cycle that require membrane fusion: uncoating and budding. (Castilla *et al.* 1998). Such chemicals might be useful therapeutically to block the spread of virus as they prevent the initial replication cycles.

The HRV genus is perhaps the most common cause of gastroenteritis with accompanying diarrhoea in infants and remains among the leading cause of early childhood death worldwide (Wagner and Hewlett 1999). Anti-HRV and anti-HSV-1 activities of hot water extracts from *Stevia rebaudi-*

Table 1 Summary of the mechanism of the most active antiviral compound from medicinal plants*

Class of compound	Mechanism virus target	Example of plant source
Furyl compounds: furocoumarins and furanochromones	DNA and RNA genomes. Interactions required long-wave ultraviolet (UVA, 300–400 nm)	Rutaceae and Umbelliferae (Apiaceae)
Alkaloids constitute: β-carbolines, furanoquinolines, camptothecin, atropine, caffeine, indolizidines swainsonine, castanospermine, colchicines, vinblastine	DNA and other polynucleotides and virions proteins. In some interactions are enhanced by UVA	Rutaceae, Camptotheca acuminate, Atropa belladona (L.), Swansona canescens, Astragalus lentiginosus, Castanospermum australe, Aglaia roxburghiana
Polyacetylenes (polyines)	Membrane interaction. Phototoxic activity frequently requires UVA	Asteraceae, Apiaceae, Campanulaceae Panax ginseng (Korean ginseng roots), Bidens sp., Chrysanthemum sibiricum
Polysaccharides	Blocking virus binding	Achyrocline flaccida, Bostrychia montagnei, Cedrela tubiflora, Prunella vulgaris, Sclerotium glucanicum, Stevia rebaudiana, Rhizophora mucronata
Thiophenes	Membrane interaction. Phototoxic activity frequently requires UVA	Aspilia, Chenactis douglasii, Dyssodia anthemidifolia, Eclipta alba, Eriophyllum lanatum
Flavonoids: amentoflavone, theaflavin, iridoids, phenylpropanoid glycosides, agathisflavone, robustaflavone, rhusflavanone, succedaneflavanone, chrysosplenol C, morin, coumarins, galangin (3,5,7-trihydroxyflavone), baicalin	Blocking RNA synthesis. Exhibited HIV-inhibitory activity	Agastache rugosa, Euphorbia grantii, Barleria prionits, Calophyllum cerasiferum, Cal. inophyllum, Cal. teysmanii, Camellia sinensis, Garcinia multiflora, Helichrysum aureonitens, Maclura cochinchinensis, Markhamia lutea, Monotes africanus, Pterocaulon sphacelatum, Rhus succedanea, Scutellaria baicalensis, Selaginella sinensis, Sophora moorcroftiana, Sophora tomentosa, Tephrosi sp.
Terpenoids: sesquiterpene, triterpenoids (moronic acid, ursolic acid, maslinic acid and saponin)	Membrane-mediated mechanisms. Inhibition of viral DNA synthesis	Acokanthera sp., Anagallis arcensis (Primulaceae), Cannabis sativa, Geum japonicum, Glycyrrhiza glabra, Glycyrrhiza radix, Glyptopetalum sclerocarpum, Gymnema sylvestre, Maesa lanceolata, Olea europa, Quillaja saponaria, Rhus javanica, Strophanthus gratus
Lignans		
Podophyllotoxin and related lignans (cyclolignanolides), such as the peltatins	Blocking virus replication	Amanoa aff. Oblongifolia, Juniperus communis, Justicia procumbens, Podophyllum peltatum
Dibenzocyclooctadiene lignans such as schizarin B and taiwanschirin D	Blocking HBV replication	Kadsura matsudai
Rhinacanthin E and rhinacanthin F	Blocking influenza virus type A replication	Rhinacanthus nasutus
Miscellaneous phenolic compounds: anthraquinone chrysophanic acid, caffic acid, eugeniin, hypericin, tannins (condensed polymers), proanthocyanidins, salicylates and quinines (naphthoquinones, naphthoquinones and anthraquinones in particular aloe emodin)	Inhibition of viral RNA and DNA replication	Aloe barbadensis, Aster scaber, Cassia angustifolia, Dianella longifolia, Euodia roxburghiana, Geum japonicum, Hamamelis virginiana, Hypericum sp, Melissa officinalis, Phyllanthus myrtifolius, Phyllanthus urinaria, Punica granatum, Rhamnus frangula, Rhamnus purshianus, Rheum officinale, Rhinacanthus nasutus, Shepherdia argentea, Syzgium aromatica, St. John's wort
Proteins and peptides		
1. Single chain ribosome-inactivating proteins	Interaction with ribosome function in the infected cell and inhibited viral protein synthesis	Clerodendrum Inerme, Dianthus caryophyllus, Gelonium multiflorum, Momordica charantia, Phytolacca Americana, Saponaria officinalis, Trichosanthes kirilowii, Triticum aestrum

	Inactivate infective HIV and HIV-infected cells	*Phytolacca Americana, Momordica charantia, Gelonum multiflorum*
Pokeweed antiviral proteins (PAP) (MRK29, MAP30 and GAP31)		
Panaxagin	Inhibit the HIV-1 reverse transcriptase	*Panax ginseng*
Alpha- and beta-antifungal proteins	Inhibit the HIV reverse transcriptase	*Vigna unguiculata*
2. Dimeric cytotoxins	Interaction with ribosome function in the infected cell and inhibit viral protein synthesis	*Ricinus communis, Abrus precatorius, Adenia digitata*
3. Lectins	Viral membrane interactions	*Canavalia ensiformis, Lens culinaris, Phaseolus vulgaris, Triticum vulgaris*
4. Antiviral factor	Mechanism of action is not known	*Nicotiana glutinosa*
5. Meliacine	Affect virus replicative cycle	*Melia azedarach*

Takechi and Tanaka (1981); Singh *et al.* (1985); Singh (1988); Hudson (1990); Sydiskis *et al.* (1991); Asano *et al.* (1996); Erdelmeier *et al.* (1996); Marchetti *et al.* (1996); McCormick *et al.* (1996); Olivieri *et al.* (1996); Pengsuparp *et al.* (1996); Sendl *et al.* (1996); Xu *et al.* (1996); Yoshida *et al.* (1996); Kernan *et al.* (1997); Meyer *et al.* (1997); Castilla *et al.* (1998); Chen *et al.* (1998); Clark *et al.* (1998); Kernan *et al.* (1998); Kurokawa *et al.* (1998a); Sindambiwe *et al.* (1998); Spino *et al.* (1998); Garcia *et al.* (1999); Kurokawa *et al.* (1999); Lin *et al.* (1999); Liu *et al.* (1999); Premanathan *et al.* (1999b); Schreiber *et al.* (1999); Semple *et al.* (1999); Sotanaphun *et al.* (1999); Xu *et al.* (1999); Alche *et al.* (2000); Bunyapraphatsara *et al.* (2000); Kwon *et al.* (2000); Li *et al.* (2000a,b); Sánchez *et al.* (2000); Ye *et al.* (2000); Zheng *et al.* (2000); Craig *et al.* (2001); D'Cruz and Uckun (2001); Duarte *et al.* (2001); Jacobson *et al.* (2001); Jiratchariyakul *et al.* (2001); Kuo *et al.* (2001); Ma *et al.* (2001); Meragelman *et al.* (2001); Ng and Wang (2001); Semple *et al.* (2001); Shirataki *et al.* (2001); Takahashi *et al.* (2001).

ana and *Achyrocline flaccida*, respectively, have been examined (Garcia *et al.* 1999; Takahashi *et al.* 2001). The extracts inhibited the replication of four serotypes of HRV and HSV-1 *in vitro* by blocking the binding of the virus. The inhibitory components of the *Stevia rebaudiana* and *Achyrocline flaccida* extracts were found to be heterogeneous anionic polysaccharides with different ionic charges. Polysaccharides extracted from the leaf of *Rhizophora apiculata* (RAP) and the bark of *Rhizophora mucronata* (RMP) were assessed by an *in vitro* cell culture system (Premanathan *et al.* 1999a,b). Both RAP and RMP protected MT-4 cells from HIV-induced cytopathogenicity and blocked the expression of HIV p24 antigens (viral capsid protein) preventing the virus binding to the cell and the formation of syncytia upon co-cultivation of MOLT-4/HIV-1IIIB cells and MOLT-4 cells.

Gauntt *et al.* (2000) found that aloe polymannose (AP), a mannose biological response modifier (BRM) purified from the *Aloe barbadensis* Miller plant, enhanced concentrations of anti-CVB3 antibodies. The data conclusively showed that AP can immunopotentiate antibody production against capsid protein epitopes of a nonenveloped picornavirus and suggests that BRM (AP) might be of benefit in enhancing antibody concentrations against other enteroviruses and poliovirus vaccine strains. More studies are needed to understand viral capsid protein function and structural properties in the presence the polysaccharide BRM that stimulates the production of antibody against nonenveloped viruses.

The mechanisms of the binding of antiviral agents to the virion, and the mechanisms of the viral binding to host cells in the presence of scleroglucan polysaccharide seems to be related to its binding with membrane glycoproteins of viral particles which then impedes the complex interactions of the virus with the cell plasma membrane (Marchetti *et al.* 1996). This is particularly important for compounds, which are known to affect membranes. Blocking of the viral attachment to host cells will prevent the productive infection cycle and the viral genome will not be introduced into the cell (Wagner and Hewlett 1999).

The bioflavonoids comprise a large family of plant-derived polyphenolic compounds of low molecular weight, which exhibit diverse biological activities. The naturally occurring benzo-δ-pyrone derivatives and phenylchromones are widely distributed throughout the plant kingdom, as components in fruits, vegetables, tea, grains, bark, roots, stems and flowers. Up to several hundred milligrams are consumed daily in the average Western diet. On balance, a considerable body of evidence suggests that plant flavonoids may be health promoting, disease-preventing dietary compounds (Middleton 1998). They are the basis of many traditional folk remedies which have many important biochemical effects, some of which have been

applied in human therapy, as described in the review by Havsteen (1983) and are now being increasingly used as prototypes for the development of specific drug therapies (Berger *et al.* 1992).

The antiviral activities of bioflavonoids extracted from medicinal plants have been evaluated (Beladi *et al.* 1977; Tsuchiya *et al.* 1985). The black tea flavonoid, theaflavin is a well-known antioxidant with free radical-scavenging activity and it was able to neutralize bovine rotavirus and bovine corona virus infections (Clark *et al.* 1998).

The flavonoid chrysosplenol C is one of a group of compounds known to be a potent and specific inhibitor of picornaviruses and rhinoviruses, the most frequent causative agents of the common cold (Semple *et al.* 1999). The *Dianella longifolia* and *Pterocaulon sphacelatum*, were found to contain flavonoid chrysosplenol C and anthraquinone chrysophanic acid, respectively, which inhibit the replication of poliovirus types 2 and 3 (*Picornaviridae*) *in vitro* (Semple *et al.* 1999, 2001; Table 1). Recently, new flavonol glycoside – the iridoid glycosides and three phenylpropanoid glycosides, named luteoside A, luteoside B and luteoside C – were isolated from *Barleria prionitis* and from the roots of the medicinal plant *Markhamia lutea*, respectively, and shown to have potent *in vitro* activity against RSV (Chen *et al.* 1998; Kernan *et al.* 1998). In another study, five groups of biflavonoids (amentoflavone, agathisflavone, robustaflavone, rhusflavanone and succedaneflavanone) were isolated from medicinal plants of *Rhus succedanea* and *Garcinia multiflora*, and exhibited various antiviral effects against a number of viruses including respiratory viruses (influenza A, influenza B, parainfluenza type 3, RSV, adenovirus type 5 and measles) and herpes viruses (HSV-1, HSV-2, HCMV and varicella zoster virus, VZV) (Lin *et al.* 1999). Amentoflavone and robustaflavone, demonstrated significant activity against anti-HSV-1 and anti-HSV-2 with only moderate anti-HSV-2 from rhusflavanone. A significant anti-influenza A and B activity was achieved by amentoflavone, robustaflavone and agathisflavone. By comparison, rhusflavanone and succedaneflavanone were found to produce a selective anti-influenza type B only. The inhibitory activities against measles and VZV were demonstrated with rhusflavanone and succedaneflavanone, respectively. In general, none of groups of biflavonoids exhibited anti-HCMV (Lin *et al.* 1999).

Baicalein (BA), a flavonoid compound purified from the medicinal plant *Scutellaria baicalensis* Georgi, has been shown to possess anti-inflammatory and anti-HIV-1 activities. BA may interfere with the interaction of HIV-1 envelope proteins with chemokine co-receptors and block HIV-1 entry of target CD4 cells and BA could be used as a basis for developing novel anti-HIV-1 agent (Li *et al.* 2000a).

Morin is another type of flavonoid group extracted from *Maclura cochinchinensis* that exhibited a powerful anti-HSV-2 activity in contrast with a synthetized morin pentaacetate that was inactive (Bunyapraphatsara *et al.* 2000). This would suggest that free hydroxyl groups are required for anti-HSV-activity, as demonstrated previously for the antiviral activity of other flavonoids (Hudson 1990; Bunyapraphatsara *et al.* 2000). Such studies clearly indicate that antiviral activity varies with the compound and the virus. It is premature to speculate further on chemical requirements, as the majority of studies that utilized different compounds were inadvertently designed to examine primarily the flavonoids inhibitory activity against viral enzymes. The mechanisms of binding the flavonoids extracted from medicinal plants received less attention. However, one stage of viral replication that may be inhibited by flavonoids is viral DNA synthesis. For example, SP-303 exhibited strong activity against herpes virus (HSV-1 and HSV-2) (Barnard *et al.* 1993). Most of the potent anti-HIV flavonoids such as BA, quercentin and myricetin have shown inhibitory activity not only against the virus-associated RT but also against cellular DNA or RNA polymerase (Ono and Nakane 1990). The fact that the RT plays a very important role in controlling the replication of HIV makes it one of the most attractive targets in the development of anti-AIDS drugs. The inhibition of DNA and RNA polymerase by these flavonoids was extensively analysed to elucidate the inhibition mechanism(s) by Ono and Nakane (1990). Once again the degree of inhibition also varied depending on the flavonoid.

The oligostilbenes isolated from the organic extract of the leaves of *Hopea malibato* was also investigated against HIV and found that a new oligostilbene dibalanocarpol, together with one known oligostilbene balanocarpol exhibited only modest HIV-inhibitory activity (Dai *et al.* 1998).

The state of Sarawak, on the island of Borneo, Malaysia, is known internationally for its rich rainforests and has attracted the attention of scientists for their potential medicinal value. Species of the *Calophyllum* tree produce active anti-HIV agents. This has intensified interest in the State's plant resources for scientific research (Chung 1996). One approach has been taken to identify novel inhibitors of HIV-1-RT by the screening of natural compounds of the *Calophyllum* tree. The most extensive screening effort, carried out by researchers was on inophyllum, calanolide A and coumarins isolated from the terrestrial plants of *Calophyllum inophyllum*, *Cal. lanigerum*, *Cal. teysmannii* latex and *Cal. cerasiferum*, respectively. They possess the most interesting natural RT inhibitor (Taylor *et al.* 1994; Currens *et al.* 1996; Pengsuparp *et al.* 1996; Spino *et al.* 1998). It was found that both inophyllum (Taylor *et al.* 1994) and calanolide A (Currens *et al.* 1996) represented a novel subclass of non-nucleoside RT inhibitor and merited consideration for anti-HIV drug development.

More than 200 lignans have been identified, and they have a widespread distribution in the plant kingdom, including many medicinal plants some of which showed promising antiviral activities (Hudson 1990). Recently, a new class of lignans isolated from *Larrea tridentates*, *Rhinacanthus nasutus* and *Kadsura matsudai* showed anti-HIV, anti-influenza and anti-hepatitis potencies, respectively. With their important clinical relevance, they do merit further investigation (Gnabre *et al.* 1996; Kernan *et al.* 1997; Li *et al.* 2000b; Kuo *et al.* 2001).

Rhus javanica has been shown to exhibit anti-HSV-2 activity and potentiate the anti-HSV activity of acyclovir *in vitro* and *in vivo* (Nakano *et al.* 1999). Moronic acid, a simple triterpenoid keto acid with antimicrobial activity (Hostettmann-Kaldas and Nakanishi 1979), purified from the herbal extract of *Rhus javanica* showed oral therapeutic efficacy with respect to wild-type HSV- (type1 and type 2) infected mice. There is no question about the efficacy of triterpenoids, in particular that of moronic acid, but it is not clear if this is due to a direct antiviral effect or whether this reflects the known healing properties of this compound in nonviral mucosal lesions (Hudson 1990). One might also suggest a role for interferon, which can be induced by triterpenoids (Hudson 1990). For example, the triterpene acids of *Geum japonicum* such as ursolic acid and maslinic acid showed potent inhibitory activity against HIV-1 protease (Xu *et al.* 1996). It may at least in part be attributed to interference with virus–cell binding, as in the case of triterpene glycyrrhizin (extracted from the licorice root *Glycyrrhiza radix*) (De Clercq 2000). Herpes infections are known to be relatively poor responders to interferon (Hudson 1990), so the question of exactly how triterpenoids work against virus infections *in vivo* remains unanswered.

The phenolic compound eugeniin (ellagitannin) extracted from *Geum japonicum* and *Syzygium aromaticum* demonstrated clearly its anti-HSV activity (Takechi and Tanaka 1981; Kurokawa *et al.* 1998a). A detailed analysis was made of viral DNA synthesis, and eugeniin was found to inhibit the growth of acyclovir-phosphonoacetic acid-resistant HSV-1, thymidine kinase-deficient HSV-1 and wild HSV type 2, and Epstein–Barr virus DNA polymerase. One of the major target sites of inhibitory action of eugeniin is viral DNA synthesis (Kurokawa *et al.* 1998a; Liu *et al.* 1999).

Different kinds of anthraquinones from extracts of *Rheum officinale*, *Aloe barbadensis* (*Aloe vera*), *Rhamnus frangula*, *Rhamnus purshianus*, and *Cassia angustifolia* were found to be quite active against HSV-1 (Sydiskis *et al.* 1991). In contrast, anthraquinones were found inactive against varicella zoster virus, pseudorabies virus, influenza virus, adenovirus, poliovirus, semliki forest virus, coxsackievirus, measles and rhinovirus (Van den Berghe *et al.* 1986; Sydiskis *et al.* 1991). Nonetheless, progress has been made

with a purified sample of aloe emodin (the common aglycones which may exist as anthraquinones), prepared from aloin. It inactivated HSV-1, varicella zoster virus, pseudorabies virus, influenza virus *in vitro*, but not adenovirus and rhinovirus (Sydiskis *et al.* 1991).

In general, the aforesaid antiviral activity is attributable to the polyphenols, rosmarinic acid, and the low-molecular glycoside-forming compounds of chlorogenic acid and caffeic acid, and their derivatives (Litvinenko *et al.* 1975).

6. PHYTO-THERAPY AND CLINICAL TRIAL

The use of medicinal plants for the treatment of viral infections arguably has been based largely on historical and anecdotal evidence. In India there are three major systems of traditional medicine, namely, the Ayurvedic, Siddha, and Unani systems that have standard treatments for clinical jaundice. These treatments consist of oral administration of one or more dried plant extracts, in the form of tablets or capsules. Other cultures in different parts of the globe also used plant extracts for the same purpose, e.g. licorice root *Glycyrhiza glabra* in China.

The most common ingredients in the Indian systems are extracts of the genus *Phyllanthus* of the Euphorbiaceae family. The plants are widely distributed in most tropical and subtropical countries, and have long been used in folk medicine to treat diabetes, kidney and urinary bladder disturbances, intestinal infections and the treatment of viral, bacterial and parasitic infections (Calixto *et al.* 1998; Sanchez-Lamar *et al.* 1999). In recent years substantial progress on chemical and pharmacological properties, as well as a few clinical studies of some *Phyllanthus* species have been made. Thyagarajan *et al.* (1988, 1990) reported that dried milled *Phyllanthus amarus* was successful in clearing hepatitis B surface antigen (HBsAg) from blood positive carriers in Madras, India. Extracts of *Phy. amarus*, standardized to contain 20 mg of geraniin per dose, had no effect on levels of HBsAg or HBeAg when given three times daily to hepatitis B carriers from New Zealand for a period of 2 months (Milne *et al.* 1994). A powder of the *Phy. amarus* plant was compared with placebo in patients with acute hepatitis B virus (HBV) (Narendranathan *et al.* 1999). Fifty-six patients were randomized to receive either the placebo (28 cases) or the drug (28 cases). The duration of the disease in the two groups was compared by Cox's proportional hazards analysis after adjusting for the variables that influence the duration of jaundice. The analysis showed that *Phy. amarus* powder did not significantly reduce the duration of jaundice in HBV persons.

In general the subsequent clinical results concerning the use of *Phyllanthus* species for hepatitis has been conflicting and this may have much to do with the extract standardization, species used and location harvested that resulted in

different levels of active constituents in samples used (Wang *et al.* 1994). Therefore, Wang *et al.* (1995) tested the effects of three different *Phyllanthus* species extracts on the serologic status of 123 patients with chronic hepatitis B. Eleven patients received an extract of *Phy. amarus* (L.) provided by S.P. Thyagarajan, Madras, India. Forty-two patients received *Phy. niruri* (L.), gathered from Hainan Province in China, and 35 patients received an extract of *Phy. urinaria* (L.), which had been gathered in Henan Province. Thirty-five control patients received no herbal therapy. The patients receiving *Phy. urinaria* (L.) were both more likely to lose detectable HBeAg from their serum and more likely to seroconvert hepatitis B e-antibody status from negative to positive than were patients given either of the other two preparations. The status of patients was not changed with respect to HBsAg.

The literature is rife with contradictory conclusions of anti-hepatitis B activity obtained from a variety of crude *Phy. amarus* extracts. In view of these discrepancies it is hardly surprising (but not explained) that *Phy. amarus* has no value as anti-HBV activity. Notwithstanding, with respect to the above reservations it is clear that some of these studies would have missed most of the 'window' period for *Phy. amarus* anti-HBV activity. This emphasizes the necessity of looking for concentration-dependant 'significant' decreases in virus concentrations. *In vitro Phy. amarus* at 1 mg ml^{-1} concentration inhibited the secretion of HBsAg for a period of 48 h. The plant suppresses HBV mRNA transcription by a specific mechanism of action involving interactions between HBV enhancer I and C/EBP alpha and beta transcription factors, which exhibit therapeutic potential in chronic HBV carriers (Lee *et al.* 1996; Ott *et al.* 1997). The disruption by *Phy. amarus* of HBV polymerase activity, mRNA transcription, and replication supports its role as an antiviral agent (Lee *et al.* 1996).

Phyllanthus niruri was also evaluated for anti-hepatitis activity (Thyagarajan *et al.* 1982; Hudson 1990) in more detail against HBsAg positive sera (i.e. from chronic hepatitis B carriers). It was found that the plant extract 'inactivated' HBsAg, the effect being faster at 37°C than at 4°C. The toxicity studies for these extracts were performed in cell cultures and in mice and it was shown that the *Phy. niruri* extract had no toxic effect on vero cells or in mice (Hudson 1990). A clinical trial was carried out with *Phy. niruri* extract on a series of HBsAg positive carriers. They were given the extract or a placebo daily for 30 days (Hudson 1990). A 90-day post-treatment has shown that approximately two-thirds of the treated positive individuals cleared their HBV antigen. A study that followed indicated that *in vivo*, *Phy. niruri* eliminated hepatitis B in mammals within 3–6 weeks (Wang *et al.* 1995). Another study examined the effects of aqueous extracts of *Phy. niruri* on the woodchuck hepatitis virus (WHV) (Venkateswaran *et al.*

1987). This virus has often been used as a model for HBV, as its pathogenesis in woodchucks appears to be similar to its human counterpart. The extract was found to inhibit the binding of both HBV and WHV surface antigens (HBsAg and WHsAg) to their corresponding antibodies. In addition the extract inhibited, in a dose-dependent manner, the WHV DNA-polymerase activity *in vitro*. These reactions could explain in part the beneficial effects of the extract in patients. Thus antigen-antibody (immune) complexes would be inhibited, and virus replication, which is normally restricted to parenchymal cells of the liver, could be blocked. The extract found to be tolerated well by mice following intraperitoneal injections. The extract was tested in virus-carrier woodchucks. Intraperitoneal inoculations resulted in a gradual but impressive decrease in WHsAg, which did not subsequently reappear. A similar result was seen in animals that had recently acquired the infection; the antigen concentration dropped dramatically and did not rise again after stopping the treatment. Furthermore, serum viral DNA-polymerase activity disappeared during treatment and did not reappear. Subsequent histology of the livers revealed only mild residual pathology in the treated animals, in contrast to the usual severe pathology seen in untreated carriers.

Thus, although the number of animals tested was quite small, there was sufficient evidence from these studies to support the belief that *Phy. niruri* extracts can act therapeutically against hepatitis B infections to halt the spread of virus and immune complexes and thus allow the restoration of normal liver histology and functions.

Hepatitis C virus (HCV) is emerging as a serious worldwide problem. The use of the botanical components glycyrrhizin, catechin, silymarin and phytosterols, and the antioxidants *N*-acetylcysteine and vitamin E were reviewed for their efficacy in treating chronic hepatitis and affecting liver damage (Patrick 1999). The potential of medicinal herbs *Acacia nilotica*, *Boswellia carterii*, *Embelia schimperi*, *Piper cubeba*, *Quercus infectoria*, *Trachyspermum ammi* and *Syzygium aromaticum* extracts were investigated *in vitro* and a significant inhibiting activity against HCV protease were reported (Hussein *et al.* 2000). More recently, five patients with chronic hepatitis C were treated for 1 year with Iscador® Spezial (Weieda, Schwabisch, Germany), the brand name of an aqueous *Viscum album* extract. The yields in HCV production was reduced about 6–20-fold in two patients along with normalization of liver function and improved life quality and there were no serious side effects (Tusenius *et al.* 2001).

The potential of phyto-therapy for treatment of HIV positive patients was studied and recently in the USA a phase I dose-escalating clinical trial of andrographolide extracted from *Andrographis paniculata* was conducted in 13 HIV positive patients and five HIV uninfected, healthy

volunteers (Calabrese *et al.* 2000). The planned regimen was 5 mg kg^{-1} bodyweight for 3 weeks, escalating to 10 mg kg^{-1} bodyweight for 3 weeks, and to 20 mg kg^{-1} bodyweight for a final 3 weeks. At the end of the trial there was a significant rise in the mean CD4$^+$ lymphocyte level of HIV subjects after administration of 10 mg kg^{-1} andrographolide. There were no statistically significant changes in mean plasma HIV-1 RNA levels throughout the trial. It was concluded that andrographolide may inhibit HIV-induced cell cycle disregulation, leading to a rise in CD4$^+$ lymphocyte levels in HIV-1 infected individuals.

It is well known that HSV is an example of a classic latent viral infection (Wagner and Hewlett 1999). A double-blind, placebo-controlled, randomized trial was carried out to treat 66 patients with a history of recurrent herpes labialis (at least four episodes per year) using a standardized balm mint cream, Lomaherpan® (Natural Medicine Research, Emmenthal, Germany), prepared from *Melissa officinalis* (L.) leaves extract (Koytchev *et al.* 1999). The cream was smeared on the affected area four times daily over 5 days. The tested formulation was found to be effective for the treatment of herpes simplex labilalis without any cytotoxic side reactions. It remains to be further investigated whether the extract of *Melissa officinalis* (L.) leaves also has a therapeutic advantage to treat infections of genital mucosa, and HSV-2, which invades the sciatic nerve ganglia.

7. THE EFFECT OF SYNERGETIC COMBINATION OF MEDICINAL PLANTS IN THE POTENCY OF ANTIVIRAL ACTIVITY AGAINST SELECTED VIRUSES

Many medicinal plants/herbs are often prescribed in composite formulae according to traditional principles of treatment as an approach to neutralize or reduce toxicity of poisonous herbs (Xu and Chan 1994). Different combinations of plants can cause variations in therapeutic effects. Related studies showed that the dry Gingyo-san used in traditional antipyretic medicine for the treatment of the common cold and influenza virus infection has significantly reduced fever production and suppressed the rise in interleukin (IL)-1 alpha caused by influenza infection (Kurokawa *et al.* 1998b). Furthermore, the latter authors have shown that of the 10 crude components of Gingyo-san, Saigae Tataricae Cornu simultaneously exhibited antipyretic and IL-1 alpha–regulatory activities. In a further study, a multicomponent herbal formula Ledretan-96 (Laboratory of Applied Pharmacology, State Institute, Staten Island, NY, USA) consisting of 23 individual components were tested on an epithelial tissue culture cell line (Madin-Darby Canine Kidney, MDCK) for its protective activity against cyto-pathic effects caused by influenza A virus (Badmaev and Nowakowski 2000). Of the 23 components tested, only one,

Terminalia chebula, showed a significant protective effect when applied to the epithelial cells individually, but over all, the synergism has indicated that the complete formula maintained antiviral activity at a higher therapeutic index than the *Terminalia chebula* extract alone. In theory at least there is a possibility of synergism between two or more components, which together could provide useful antiviral activity (Hudson 1990). This may explain the success of many medicinal plant extracts, which could be therapeutic-ally useful for several apparently unrelated syndromes by virtue of the synergistic effects of two or more components that complement each other *in vivo*. It is also likely that some of the ingredients have restorative/activation functions, although these have not yet been defined.

A chemical activation of antiviral activity from pomegranate (*Punica granatum* L.) rind, *Viburnum plicatum* (leaves or flowers), *Camellia sinensis* (tea leaves) or *Acer pseudoplatanus* (maple leaves) extracts was reported by using FeSO$_4$·7H$_2$O (Jassim *et al.* 1995; Stewart *et al.* 1998). Furthermore, the results by PCR revealed that this novel approach to restore functions of antiviral activity have resulted in the cleavage of viral RNA/DNA (Jassim *et al.* 1995).

8. ASPECTS OF SYNERGETIC COMBINATION OF MEDICINAL PLANTS WITH ORTHODOX DRUGS FOR ALLEVIATING VIRAL INFECTION

It is observed that patients in the Far East intentionally or unintentionally are incorporating orthodox medical drugs into herbal medicinal preparations for alleviating their illnesses (Chan and Cheung 2000). The rationale for doing so is to reduce the side effects of orthodox medical drugs, and to produce synergistic effects for better treatment outcome. It has become apparent that not many of these combinations are successful. Some of the over-the-counter medicinal plant products containing orthodox medical drugs are available to the public. In most cases the pharmacological mechanisms of the combinations are not well-studied and exaggerated adverse effects or therapeutic failures have been observed (Chan and Cheung 2000), although successful treatments using combination of medicinal plant products with orthodox drugs were also reported. In a clinical study Corina *et al.* (1999) examined the effect of extractants of Romanian medicinal plants in combination with acyclovir in the treatment of 52 patients suffering herpetic keratitis. Better results and faster healing of ulceration were obtained using *Actium lappa*, *Calendula officinalis* and *Geranium robertianum* extracts then with the usual acyclovir treatment only. Amantadine hydrochloride is an accepted and well-studied selective inhibitor of influenza virus reproduction. Recently, a combined application of flowers of *Verbascum thapsiforme* (Scrophulariaceae) (Flos Verbasci infusion, FVI)

and three amantadine derivatives resulted in a marked enhancement of the inhibitory effect of FVI on the reproduction of influenza virus (Serkedjieva 2000b).

9. FUTURE PROSPECTS

Today viral diseases are still fatal, although some can be kept under control with life-prolonging drugs. These expensive antiviral drugs are still far beyond the means of most developing countries. Arguably, the development of safe, effective and inexpensive antiviral drugs active as RT inhibitors is among the top global priorities of drug development, as many viruses are not yet curable and mortality rates are high, for example with HIV and hepatitis. Furthermore, the long-term efficacy of new combination drug therapies for HIV infection may be limited by the tendency of transfected HIV to mutate to drug-resistant forms. Therefore, it is essential to continue the search for useful and novel natural antiviral agents, which can be expected to prolong the efficacy of drug therapy in subjects infected with HIV. Recently, considerable attention has been given to screening of various species of medicinal plant extracts for possible anti-HIV activity (Chang and Yeung 1988; Taylor et al. 1994; Jassim et al. 1995; Currens et al. 1996; Pengsuparp et al. 1996; Spino et al. 1998; Yamasaki et al. 1998; Hussein et al. 1999; Premanathan et al. 1999a,b; Calabrese et al. 2000; Asres et al. 2001; Motohashi et al. 2001).

There are many parameters that should be taken into consideration in the evaluation of anti-HIV activity extracted from medicinal plants, for example the methods of extraction used since the greatest degree of antiviral activity against both HIV-1 and HIV-2 was achieved with the acetone extract of *Combretum paniculatum*, whilst, the methanol fraction of *Dodonaea angustifolia* showed only selective activity toward the HIV-1 (Asres et al. 2001). There are also well-known three different pokeweed anti-viral protein (PAP) isoforms from leaves of *Phytolacca Americana* (PAP-I from spring leaves, PAP-II from early summer leaves, and PAP-III from late summer leaves) that cause concentration-dependent depurination of genomic HIV-1 RNA (Rajamohan et al. 1999). Therefore at the outset of a screening programme involving medicinal plants, it is wise to identify the exact method of preparation of an extract, the parts of the plant to be used, the appropriate season(s) for gathering the materials and the details of administration (Hudson 1990).

Many plants contain ribosome-inactivating proteins (RIPs) that alter ribosomal function in the infected cell and inhibit viral protein synthesis (Table 1; Olivieri et al. 1996). It was found that RIPs are toxic N-glycosidases that depurinate the universally conserved alpha-sarcin loop of large rRNAs and that enzymatic activity of at least some

RIPs is not limited to site-specific action on the large rRNAs of ribosomes but extends to depurination and even nucleic acid scission of other targets (Wang and Tumer 1999; Nielsen and Boston 2001). This depurination inactivates the ribosome, thereby blocking its further participation in protein synthesis. For example, the substance trichobitacin is an RIP purified from the root tubers of *Trichosanthes kirilowii* that was found to markedly reduce both expression of HIV-1 p24 antigen and the number of HIV antigen positive cells in acutely but not chronically HIV-1 infected culture (Zheng et al. 2000). The molecular mechanism of the PAP was investigated by directly measuring the amount of adenine released from the viral RNA species using quantitative high-performance liquid chromatography (Rajamohan et al. 1999). It was found that PAP29 (*Phytolacca* anti-HIV protein; molecular weight, 29 kDa) is another single-chain RIP purified from leaves of *Phytolacca Americana*. It has a particular clinical usefulness similar to MAP30 (Momordica anti-HIV protein; molecular weight: 30 kDa) and GAP31 (Gelonium anti-HIV protein; molecular weight: 31 kDa), obtained from *Momordica charantia* and *Gelonium multiflorum*, respectively (Schreiber et al. 1999), as a prophylactic anti-HIV agent. It can inactivate infective viruses and virus-infected cells in semen with nonspermicidal intravaginal microbicide (D'Cruz and Uckun 2001).

The first report of a clinical pharmacokinetic study of TXU (anti-CD7)-PAP immunoconjugate in HIV-infected patients was reported (Uckun et al. 1999) with superior *in vitro* anti-HIV-1 activity of PAP compared with the activity of zidovudine (Uckun et al. 1998). These observations may provide the basis for further investigation of PAP as a potential biotherapeutic agent for HIV patients.

Phyllanthus niruri has potential beneficial therapeutic actions in the management of hepatitis B (Calixto et al. 1998), and its antiviral activity extends to HIV-1 RT inhibition (Ogata et al. 1992). Qian-Cutrone et al. (1996) isolated from a dried leaf of *Phy. niruri*. A novel compound, which they named 'niruriside', has a specific inhibitory activity against the binding of a regulator of expression of the virion (REV) protein to REV-response element (RRE) RNA. The REV-RRE regulatory mechanism plays a key role in the maintenance of high levels of viral propagation (Von Gegerfelt et al. 2002). Therefore, it can be further suggested that a partial block of REV function of *Phy. niruri* may modulate progression in HIV-infected individuals.

It is well known that proteolytic viral enzymes play a key role in triggering disease (Jassim and Naji 2001). Interestingly, aqueous extracts of *Acacia nilotica* (pods) and *Maytenus senegalensis* (stem-bark) showed considerable inhibitory effects against HIV-1 protease (Hussein et al. 1999). If viral enzymes could be neutralized, viral replication could not take place. As the virus must have the infected cell

to translate its genetic information into proteins, it must be able to express mRNA in the infected cell. With negative RT activity the viral proliferation will not take place. Developing specific inhibitors for viral protease activity from medicinal plants are desirable objectives.

10. CONCLUSION

Many traditional medicinal plants and herbs were reported to have strong antiviral activity. Aqueous and organic extractions have in general proved equally fruitful; thus it is not feasible at present to assert which method of extraction is preferable.

In view of the signification number of plant extracts that have yielded positive results it seems reasonable to conclude that there are probably numerous kinds of antiviral agents in these materials. Further characterization of the active ingredients will reveal useful compounds. Some of these compounds belong to a wide range of different structural classes, e.g. coumarins, flavonoids, tannins, alkaloids, lignans, terpenes, naphtho- and anthraquinones, polysaccharides, proteins and peptides. Others may turn out to be identical to, or structurally related to, the antivirals, which are illustrated in Table 1. There may also be novel phytochemicals. Although large numbers of new compounds have been isolated from medicinal plants only some have been marketed as pharmaceutical products. Some compounds have been or are undergoing various phases of clinical trials.

The traditional use of some of the medicinal plants for the treatment of infectious diseases of viral origin, therefore, is justified.

Finally, the development of new medicinal plant products is vital in controlling the threats posed by some pathogenic viruses. There is little likelihood that available orthodox antiviral drugs can eliminate all or even most viral diseases.

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