ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Review Article

REVIEW ON ANTIVIRAL AND IMMUNO-STIMULATORY POTENTIAL OF MEDICINAL PLANTS

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ABSTRACT

Research on medicinal plants has attracted a lot of attention globally in past few years. A wide variety of active phytochemicals, including the flavonoids, terpenoids, lignans, sulphides, polyphenolics, coumarins, saponins, furyl compounds, alkaloids, polyines, thiophenes, proteins and peptides of plants and herbs have been identified for its medicinal and antiviral properties. 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 non randomized crossovers and pre and post-treatment analysis. Methods are needed to link antiviral efficacy or potency and laboratory based research. However, 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. In this review we illustrates, 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, Poxviridae, Reoviridae and Herpesviridae.

KEYWORDS: Antiviral agent, Medicinal Plants, Herbs, Phytochemicals, Retroviridae, HIV, RSV.

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[58]. Unlike bacterial cells, which are free-living entities, viruses utilize the host cell environment to propagate new viruses. Viruses that spread easily, kill sometimes swiftly, and for which there is no cure or vaccine that's why, in recent years, research on medicinal plants has attracted a lot of attention globally[39]. Like any other kind of infection, control of viral infection 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[58]. 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[58]. 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 protease is essential for maturation of the virus. Designing specific inhibitors for each of viral protease is thus a desirable objective for researchers now a days. Many plants are rich in a wide variety of secondary metabolites such as tenins, terpinoids, phenol, alkaloids, and flavonoids etc which have been found to be potent antiviral properties. 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 human activity and civilization. We have barely scraped the surface in our efforts to exploit the plant world for antimicrobials (namely, antiviral, antibacterial and antifungal compounds) and immunostimulatory action of phytochemicals. 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.

History of Antiviral Medicinal Plants

Medicinal plant was used for health care, date back to the origin of human civilization on the earth. But 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[10]. In India, the sale of total herbal products is estimated at $ 1 billion and the export of herbal crude extract is about $ 80 million, of which 50% is contributed by Ayurvedic classical preparations.

Extraction Methods

Advice abounds for the amateur herbalist on how to prepare healing compounds from plants and herbs. Water is almost universally the solvent used to extract activity Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found. Initial screenings of plants for possible antimicrobial activities typically begin by using crude aqueous or alcohol extractions and can be followed by various organic extraction methods. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. In fact, many studies avoid the use of aqueous fractionation altogether. The exceptional water-soluble compounds, such as poly-saccharides (e.g., starch) and polypeptides, including fabatin and various lectins, are commonly more effective as inhibitors of pathogen (usually virus) adsorption and would not be identified in the screening techniques commonly used. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents. Any part of the plant may contain active components. For instance, the roots of ginseng plants contain the active saponins and essential oils, while eucalyptus leaves are harvested for their essential oils and tannins. Some trees, such as the balsam poplar, yield useful substances in their bark, leaves, and shoots.

For alcoholic extractions, plant parts are dried, ground to a fine texture, and then soaked in methanol or ethanol for extended periods. The slurry is then filtered and washed, after which it may be dried under reduced pressure and re dissolved in the alcohol to a determined concentration. When water is used for extractions, plants are generally soaked in distilled water, blotted dry, made into slurry through blending, and then strained or filtered. The filtrate can be centrifuged (approximately 20,000 3 g, for 30 min) multiple times for clarification. Crude products can then be used in disc diffusion and broth dilution assays to test for antifungal and antibacterial properties and in a variety of assays to screen for antiviral activity, as described below. Natural-products chemists further purify active chemicals from crude extracts by a variety of methods. Petalostemumol, a flavanol from purple prairie clover, was obtained from the ethanol extract by partitioning between ethyl acetate and water, followed by partitioning between n-hexane and 10% methanol. The methanol fraction was chromatographed and eluted with toluene. Terpenoid lactones have been obtained by successive extractions of dried bark with hexane, $CHCl_3$, and methanol, with activity concentrating in the $CHCl_3$ fraction. The chemical structures of the purified material can then be analyzed. Techniques for further chemical analysis include chromatography, bio autography, radioimmunoassay, various methods of structure identification, and newer tools such as fast atom bombardment mass spectrometry, tandem mass spectroscopy, high-performance liquid chromatography, capillary zone electrophoresis, nuclear magnetic resonance spectroscopy, and X-ray cystallography.

Efficacy Test in Vitro Experiments

Several methods are available to detect either virucidal or inhibitory (antiviral) plant activity. Investigators can look for cytopathic effects or plaque formation or for transformation or proliferative effects on cell lines. Viral replication may be assayed by detection of viral products such as DNA, RNA, or polypeptides. The method for assaying antiviral substances used in various laboratories is not standardized, and therefore the results are often not comparable to one another. These authors also point out that researchers must distinguish between merely toxic effects of agents on host cells and true antiviral properties of the plant extracts. It should be mentioned here that antiviral assays often screen for active substances which prevent adsorption of the microorganism to host cells; this activity is overlooked in screening procedures for antibacterial and antifungal substances.

Marine Herbs Antiviral Activity

Natural product research is increasingly turning to marine herbs as a source of natural products and is currently in preclinical and clinical evaluation. 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[3,47]. In addition, the water-soluble fraction of *Haslea ostrearia* has delayed HIV- 1-induced syncitia formation on MT4 cells[3]. 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[15]. 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[15]. It is well known that the presence of the sulphate group is necessary for antiviral activity, and potency increases with the degree of sulphation[44].

Plants with Immunostimulatory Action

Sambucol, a product isolated from *Sambucus nigra L.*, which is effective against various strains of influenza

virus and boost immune response by secreting IL-1, IL-6, IL-8, TNF-alpha[4]. The roots extract of medicinal plants *Heracleum maximum*, having antiviral activity along with immunostimulatory action as it stimulate IL-6 production in the macrophage activation assay.[60] 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 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. 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.[13]

Terpenoids And Essential Oils

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[45]. The activities of anti-herpes components could be the result of terpinen-4-ol[13]. Italian medicinal plants and food medicines were reviewed[40] 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[16]. 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[2]. Recently the antiviral effect of black seed oil (BSO) from *Nigella sativa* was investigated using murine cytomegalovirus (MCMV) as a model. Their results show that BSO exhibited a striking antiviral effect against MCMV infection, which may be mediated by increasing one's innate immunity. *Rhus javanica* has been shown to exhibit anti-HSV-2 activity and potentiate the anti-HSV activity of acyclovir in vitro and in vivo[35]. Moronic acid, a simple triterpenoid keto acid with antimicrobial activity[21]. 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[22]. One might also suggest a role for interferon, which can be induced by triterpenoids[22]. For example, the triterpene acids of *Geum japonicum* such as ursolic acid and maslinic acid showed potent inhibitory activity against HIV-1 protease[61]. It may at least in part be attributed to interference with virus–cell binding, as in the case of triterpene glycyrrhizin[15] (extracted from the licorice root *Glycyrrhiza radix*). Herpes infections are known to be relatively poor responders to interferon[22], so the question of exactly how triterpenoids work against virus infections in vivo remains unanswered.

Tennins

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[17] and HIV-1 reverse transcriptase (RT).[62] 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.[62]

Phenolics and Polyphenols

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[7]. viral inactivation in vitro is directly attributable to preferential binding of the polyphenol to the protein coat of the virus[22] whereas, in a systematic study of the antiviral activity of a very wide range of natural products 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[56] and may exert their antiviral action[43]. 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. Polyphenols and the proanthocyanidins extracted from *Hamamelis virginiana* bark showed a remarkable inhibitory activity against HSV-1[17] and HIV-1 reverse transcriptase (RT).[62]

Peptides and Polypeptides

A peptide isolated from the leaves of the Argentinean plant *Melia azedarach* has a peptide was evaluated with mice inoculated with HSV-1 strain[1]. Infected animals treated or not with meliacine were observed carefully for the development of stromal keratitis and the clinical scoring was followed 14 days post infection. 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[9]. It also inhibited the multiplication of foot and mouth disease virus in BHK- 21 cells[57]. 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[9,57]. Taken together, these results suggest that meliacine affects two events of the virus replicative cycle that require membrane fusion: uncoating and budding[9]. Such chemicals might be useful therapeutically to block the spread of virus as they prevent the initial replication cycles. Previously, it has been shown that 1-cinnamoyl-3,11-dihydroxymeliacarpin (CDM), a natural compound isolated from leaf extracts of *Melia azedarach L.,* inhibits the vesicular stomatitis virus (VSV) multiplication cycle when added before or after infection. Here, we have established that the lack of VSV protein synthesis in CDM pre-treated Vero cells is ascribed to the inhibition of an initial step during virus multiplication, although indirect immunofluorescence (IFI) studies confirmed that the binding and uptake of [35S] methionine -labelled VSV was not affected by CDM pre-treatment. Instead, our findings revealed that this compound impedes the uncoating of VSV nucleocapsids in pre-treated Vero cells, since the antiviral action of CDM was partially reversed by inducing VSV direct fusion at the plasma membrane, and VSV M protein fluorescence was confined to the endosomes, even 2 h post-internalization. Furthermore, CDM induced cytoplasmic alkalinization, as shown by acridine orange staining, consistent with the inhibition of virus uncoating. Although VSV proteins are synthesized when CDM is added after infection, IFI studies revealed that G protein was absent from the surface of infected cells and co-localized with a Golgi marker. Therefore, CDM inhibits the transport of G protein to the plasma membrane. Taken together, these findings indicate that CDM exerts its antiviral action on the endocytic and exocytic pathways of VSV by pre or post-treatment, respectively. The phenolic compound eugeniin (ellagitannins) extracted from *Geum japonicum* and *Syzygium aromaticum* demonstrated clearly its anti-HSV activity.[27-28] A detailed analysis was made of viral DNA synthesis, and eugeniin was found to inhibit the growth of acyclovir-phosphonoaceticacid-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.[27-28, 32]

Polysaccharides

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[58]. Anti-HRV and anti- HSV-1 activities of hot water extracts from *Stevia rebaudiana* and *Achyrocline flaccida*, respectively, have been examined.[18, 50] 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[41-42]. 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. 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[33]. 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[58].

Flavonoids and Flavonols

The bioflavonoid comprise a large family of plant derived polyphenolic compounds of low molecular weight, which exhibit diverse biological activities. The naturally occurring benzopyrone 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[34]. They are the basis of many traditional folk remedies which have many important biochemical effects, some of which have been applied in human therapy[20] and are now being increasingly used as prototypes for the development of specific drug therapies[6]. The antiviral activities of bioflavonoids extracted from medicinal plants have been evaluated[5,53]. 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.[12] 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.[46] 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 & 3 in vitro[46]. 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[25]. In another study, five groups of biflavonoids (amentoflavone, agathisflavone, robustaflavone, rhus-flavanone 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, para influenza type 3, RSV, adenovirus type 5 and measles) and herpes viruses (HSV-1, HSV-2, HCMV and varicella zoster virus, VZV)[31]. 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[31]. 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[30]. 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 mechanisms[36]. 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.

Coumarins

Species of the *Calophyllum* tree produce active anti-HIV agents. This has intensified interest in the State's plant resources for scientific research[11]. 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[14,38,48,52]. It was found that both inophyllum[52] and calanolide A[14], a novel subclass of nonnucleoside RT inhibitor and merited consideration for anti-HIV drug development.

Lignans

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[22]. 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.[29,30]

Anthraquinones

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[49]. In contrast, anthraquinones were found inactive against varicella zoster virus, pseudorabies virus, influenza virus, adenovirus, poliovirus, semliki forest virus, coxsackievirus, measles and rhinovirus[49,56]. 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[49]. Antiviral activity of a crude hot glycerine extract of Aloe vera gel which was grown in Bushehr (Southwest of Iran) against HSV-2 replication in Vero cell line. The extract showed antiviral activity against HSV-2 not only before attachment and entry of virus to the Vero cells but also on post attachment stages of virus replication. The IC50 before attachment and entry of virus to the cells is 428 µg/ml and the CC50 value which is the cytotoxicity of the extract for Vero cells is 3238 µg/ml, while the calculated selectivity index (SI) is 7.56. Also, IC50 of extract on post attachment stages of replication is 536 µg/ml and the SI value for inhibition of the post attachment stages of HSV-2 replication is 6.04. Therefore, compounds of Aloe vera from Bushehr could be a good candidate as a natural source for antiviral drug development against HSV-2.[24]

Miscellaneous

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[22,59]. An aqueous preparation from *Acacia arabica var. indica Benth* (Mimosaceae) (locally known as "babul") leaves (BExt) was assessed for its in vitro antiviral activity using peste des petits ruminants virus (PPRV) as a test model in the Vero cell system. Cytopathic effect (CPE) inhibition, virus titration, cell ELISA, sandwich-ELISA (s-ELISA), and PCR assays were used to determine the antiviral effects (at maximum noncytotoxic concentrations 150 and 200 µg/mL) against PPRV, and in vitro cytotoxicity assays established the relative safety concentration of the BExt for the cells. BExt inhibited viral infectivity drastically in terms of decreased virus titer and antigen load in a dose-dependent manner either when added to cell monolayers pos tinfection or when pre incubated with virus before adsorption on the cells. Inhibition of cell-free and cell-associated PPRV during replication in presence of BExt in Vero cells, using a multistep growth curve experiment, were assessed by s-ELISA. BExt (200 µg/mL) completely inhibited PPRV replication in Vero cells that were infected with PPRV at 0.01 multiplicity of infection. Incubation of PPRV with BExt (150 and 200 µg/mL) followed by infection had a virucidal effect on subsequent progeny virus yield by a 3 log10 TCID50 reduction. This indicates that active principle(s) of BExt either inactivated the virus or inhibited the viral release. Real-time PCR data based on nucleoprotein gene showed 196.7- and 770.6-fold reduction in the viral load in the presence of BExt

concentrations of 150 and 200 µag/mL, respectively, indicating the efficacy of BExt in inhibiting PPRV multiplication. These data suggest that extracts of *A. arabica* could be a potential natural antiviral agent for management of PPR disease and also a possible addition in the traditional phyto-antiviral repertoire for viral disease control[55]. 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[37]. 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[23]. 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[54]. 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[8]. The planned regimen was 5 mg kg)1 body weight 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[58]. 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[26]. 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.

CONCLUSION

Many traditional medicinal plants and herbs were reported to have strong antiviral activity as well as immune-stimulatory 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. 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 against which neither vaccine (prophylaxis) nor therapeutics are available.

REFERENCES

1. Alche, L.E., Berra, A., Veloso, M.J. and Coto, C.E. (2000) Treatment with meliacine, a plant derived antiviral, prevents the development of herpetic stromal keratitis in mice. Medical Virology 61, 474–480.
2. Benencia, F. and Courreges, M.C. (1999) Antiviral activity of sandalwood oil against herpes simplex viruses-1 and -2. Phytomedicine 6, 119–123.
3. Berge, J.P., Bourgougnon, N., Alban, S., Pojer, F., Billaudel, S., Chermann, J.C., Robert, J.M. and Franz, G. (1999) Antiviral and anticoagulant activities of a water-soluble fraction of the marine diatom Haslea ostrearia. Planta Medica 65, 604–609.
4. Bark.V.et al.,2001. The effect of sambucol, a black elderberry, natural product, on the production of cytokines., Euk. Cytokines netw. 12(2),29-296.
5. Beladi, I., Pusztai, R., Mucsi, I., Bakay, M. and Gabor, M. (1977) Activity of some flavonoids against viruses. Annuals of the New York Academy of Sciences 284, 358–364.
6. Berger, M.E., Golub, M.S., Chang, C.T., Al-Kharouf, J.A., Nyby, M.D., Hori, M., Brickman, A.S. and Tuck, M.L. (1992) Flavonoid potentiation of contractile responses in rat blood vessels. The Journal of Pharmacology and Experimental Therapeutics 263, 78–83.
7. Cadman, C.H. (1960) Inhibition of plant virus infection by tannins. In Phenolics in Plants in Health and Disease ed. Pridham, J.B. pp. 101– 105. Oxford and London: Pergammon Press.
8. Calabrese, C., Berman, S.H., Babish, J.G., Ma, X., Shinto, L., Dorr, M., Wells, K., Wenner, C.A., et al. (2000) A phase I trial of andrographolide in HIV positive patients and normal volunteers. Phytotherapy Research 14, 333–338.
9. Castilla, V., Barquero, A.A., Mersich, S.E. and Coto, C.E. (1998) In vitro anti-Junin virus activity of a peptide isolated from Melia azedarach L. leaves. International Journal of Antimicrobial Agents 10, 67–75.
10. Chantrill, B.H., Coulthard, C.E., Dickinson, L., Inkley, G.W., Morris, W. and Pyle, A.H. (1952) The action of plant extracts on a bacteriophage of Pseudomonas pyocyanea and on influenza A virus. Journal of General Microbiology 6, 74–84.

11. Chung, F.J. (1996) Interests and policies of the state of Sarawak, Malaysia regarding intellectual property rights for plant derived drugs. Journal of Ethno pharmacology 51, 201–204.
12. Clark, K.J., Grant, P.G., Sarr, A.B., Belakere, J.R., Swaggerty, C.L., Phillips, T.D. and Woode, G.N. (1998) An in vitro study of theaflavins extracted from black tea to neutralize bovine rotavirus and bovinecoronavirus infections. Veterinary Microbiology 63, 147–157.
13. Cox, S.D., Mann, C.M. and Markham, J.L. (2001) Interactions between components of the essential oil of Melaleuca alternifolia. Journal of Applied Microbiology 91, 492–497.
14. Currens, M.J., Gulakowski, R.J., Mariner, J.M., Moran, R.A., Buckheit, R.W. Jr, Gustafson, K.R., McMahon, J.B. and Boyd, M.R. (1996) Antiviral activity and mechanism of action of calanolide A against the human immunodeficiency virus type-1. The Journal of Pharmacology and Experimental Therapeutics 279, 645–651.
15. De Clercq, E. (2000) Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection. Medical Research Review 20, 323–349.
16. De Logu, A., Loy, G., Pellerano, M.L., Bonsignore, L. and Schivo, M.L. (2000) Inactivation of HSV-1 and HSV-2 and prevention of cell-to-cell virus spread by Santolina insularis essential oil. Antiviral Research 48, 177–185.
17. Erdelmeier, C.A., Cinatl, J. Jr, Rabenau, H., Doerr, H.W., Biber, A. and Koch, E. (1996) Antiviral and antiphlogistic activities of Hamamelis virginiana bark. Planta Medica 62, 241–245.
18. Garcia, G., Cavallaro, L., Broussalis, A., Ferraro, G., Martino, V. and Campos, R. (1999) Biological and chemical characterization of the fraction with antiherpetic activity from Achyrocline flaccida. Planta Medica 65, 343–346.
19. Gauntt, C.J., Wood, H.J., McDaniel, H.R. and McAnalley, B.H. (2000) Aloe polymannose enhances anti-coxsackievirus antibody titres in mice. Phytotherapy Research 14, 261–266.
20. Havsteen, B. (1983) Flavonoids, a class of natural products of high pharmacological potency. Biochemical Pharmacology 32, 1141–1148.
21. Hostettmann-Kaldas, M. and Nakanishi, K. (1979) Moronic acid, a simple triterpenoid keto acid with antimicrobial activity isolated from Ozoroa mucronata. Planta Medica 37, 358–360.
22. Hudson, J.B. (1990) Antiviral Compounds from Plants. Boca Raton, Ann Arbor, Boston: CRC Press.
23. Hussein, G., Miyashiro, H., Nakamura, N., Hattori, M., Kakiuchi, N. and Shimotohno, K. (2000) Inhibitory effects of Sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. Phytotherapy Research 14, 510–516.
24. Keivan Zandi, Moloud Abbas Zadeh, Kohzad Sartavi and Zahra Rastian, Antiviral activity of Aloe vera against herpes simplex virus type 2: An in vitro study African Journal of Biotechnology Vol. 6 (15), pp. 1770-1773, 6 August 2007.
25. Kernan, M.R., Amarquaye, A., Chen, J.L., Chan, J., Sesin, D.F., Parkinson, N., Ye, Z., Barrett, M., et al. (1998) Antiviral phenylpropanoid glycosides from the medicinal plant Markhamia lutea. Journal of Natural Products 61, 564–570.
26. Koytchev, R., Alken, R.G. and Dundarov, S. (1999) Balm mint extract (Lo-701) for topical treatment of recurring herpes labialis. Phytomedicine 6, 225–230.
27. Kurokawa, M., Hozumi, T., Basnet, P., Nakano, M., Kadota, S., Namba, T., Kawana, T. and Shiraki, K. (1998a) Purification and characterization of eugeniin as an anti-herpesvirus compound from Geum japonicum and Syzygium aromaticum. Journal of Pharmacology and Experimental Therapeutics 284, 728–735.
28. Kurokawa, M., Basnet, P., Ohsugi, M., Hozumi, T., Kadota, S., Namba, T., Kawana, T. and Shiraki, K. (1999) Anti-herpes simplex virus activity of moronic acid purified from Rhus javanica in vitro and in vivo. Journal of Pharmacology and Experimental Therapeutics 289, 72–78.
29. Li, B.Q., Fu, T., Dongyan, Y., Mikovits, J.A., Ruscetti, F.W. and Wang, J.M. (2000a) Flavonoid baicalin inhibits HIV-1 infection at the level of viral entry. Biochemical and Biophysical Research Communications 276, 534–538.
30. Li, S.Y., Wu, M.D., Wang, C.W., Kuo, Y.H., Huang, R.L. and Lee, K.H. (2000b) A novel anti-HBeAg homolignan, taiwanschirin D from Kadsura matsudai. Chemical and Pharmaceutical Bulletin (Tokyo) 48, 1992–1993.
31. Lin, Y.M., Flavin, M.T., Schure, R., Chen, F.C., Sidwell, R., Barnard, D.L., Huffman, J.H. and Kern, E.R. (1999) Antiviral activities of biflavonoids. Planta Medica 65, 120–125.
32. Liu, K.C., Lin, M.T., Lee, S.S., Chiou, J.F., Ren, S. and Lien, E.J. (1999) Antiviral tannins from two Phyllanthus species. Planta Medica 65, 43–46.
33. Marchetti, M., Pisani, S., Pietropaolo, V., Seganti, L., Nicoletti, R., Degener, A. and Orsi, N. (1996) Antiviral effect of a polysaccharide from Sclerotium glucanicum towards herpes simplex virus type 1 infection. Planta Medica 62, 303–307.
34. Middleton, E. Jr (1998) Effect of plant flavonoids on immune and inflammatory cell function. Advances in Experimental Medicine and Biology 439, 175–182.
35. Nakano, M., Kurokawa, M., Hozumi, T., Saito, A., Ida, M., Morohashi, M., Namba, T., Kawana, T. et al. (1999) Suppression of recurrent genital herpes simplex virus type 2 infection by Rhus javanica in guinea pigs. Antiviral Research 41, 153–154.
36. Ono, K. and Nakane, H. (1990) Mechanisms of inhibition of various cellular DNA and RNA polymerases by several flavonoids. Journal of Biochemistry (Tokyo) 108, 609–613.
37. Patrick, L. (1999) Hepatitis C: epidemiology and review of complementary/ alternative medicine treatments. Alternative Medicine Review 4, 220–238.
38. Pengsuparp, T., Serit, M., Hughes, S.H., Soejarto, D.D. and Pezzuto, J.M. (1996) Specific inhibition of human immunodeficiency virus type 1 reverse transcriptase mediated by soulattrolide, a coumarin isolated from the latex of Calophyllum teysmannii. Journal of Natural Products 59, 839–842.
39. Peter, J. (1994) Viruses: on the edge of life, on the edge of death. National Geographic Magazine July, 58–91.
40. Pieroni, A. (2000) Medicinal plants and food medicines in the folk traditions of the upper Lucca Province, Italy. Journal of Ethnopharmacology. 70, 235–273.
41. Premanathan, M., Arakaki, R., Izumi, H., Kathiresan, K., Nakano, M., Yamamoto, N. and Nakashima, H. (1999a) Antiviral properties of a mangrove plant, Rhizophora

apiculata Blume, against human immunodeficiency virus. Antiviral Research 44, 113–122.
42. Premanathan, M., Kathiresan, K., Yamamoto, N. and Nakashima, H. (1999b) In vitro anti-human immunodeficiency virus activity of polysaccharide from Rhizophora mucronata Poir. Bioscience, Biotechnology and Biochemistry 63, 1187–1191.
43. Sakagami, H., Sakagami, T. and Takeda, M. (1995) Antiviral properties of polyphenols. Polyphenol Actualites 12, 30–32.
44. Schaeffer, D.J. and Krylov, V.S. (2000) Anti-HIV activity of extracts and compounds from algae and cyanobacteria. Ecotoxicology and Environmental Safety 45, 208–227.
45. Schnitzler, P., Schon, K. and Reichling, J. (2001) Antiviral activity of Australian tea tree oil and eucalyptus oil against herpes simplex virus in cell culture. Die Pharmazie 56, 343–347.
46. Semple, S.J., Pyke, S.M., Reynolds, G.D. and Flower, R.L. (2001) In vitro antiviral activity of the anthraquinone chrysophanic acid against poliovirus. Antiviral Research 49, 169–178.
47. Serkedjieva, J. (2000a) Antiherpes virus effect of the red marine alga Polysiphonia denudata. Zeitschrift fuer Naturforchung. Section C 55, 830–835.
48. Spino, C., Dodier, M. and Sotheeswaran, S. (1998) Anti-HIV coumarins from Calophyllum seed oil. Bioorganic and Medicinal Chemistry Letters 8, 3475–3478.
49. Sydiskis, R.J., Owen, D.G., Lohr, J.L., Rosler, K.H. and Blomster, R.N. (1991) Inactivation of enveloped viruses by anthraquinones extracted from plants. Anti-microbial Agents and Chemotherapy 35, 2463–2466.
50. Takahashi, K., Matsuda, M., Ohashi, K., Taniguchi, K., Nakagomi, O., Abe, Y., Mori, S., Sato, N., et al. (2001) Analysis of antirotavirus activity of extract from Stevia rebaudiana. Antiviral Research 49, 15–24.
51. Takechi, M. and Tanaka, Y. (1981) Purification and characterization of antiviral substance from the bud of Syzgium aromatica. Planta Medica 42, 69–74.
52. Taylor, P.B., Culp, J.S., Debouck, C., Johnson, R.K., Patil, A.D., Woolf, D.J., Brooks, I. and Hertzberg, R.P. (1994) Kinetic and mutational analysis of human immunodeficiency virus type 1 reverse transcriptase inhibition by inophyllums, a novel class of nonnucleoside inhibitors. Journal of Biological Chemistry 269, 6325–6331.
53. Tsuchiya, Y., Shimizu, M., Hiyama, Y., Itoh, K., Hashimoto, Y., Nakayama, M., Horie, T. and Morita, N. (1985) Antiviral activity of natural occurring flavonoids in vitro. Chemical and Pharmaceutical Bulletin (Tokyo) 33, 3881–3886.
54. Tusenius, K.J., Spoek, J.M. and Kramers, C.W. (2001) Iscador Qu for chronic hepatitis C: an exploratory study. Complementary Therapies in Medicine 9, 12–16.
55. V. Balamurugan, A. Sen, P. Saravanan, V. Bhanuprakash, R. C. Patra, D. Swamp, Mahesh Kumar d, M. P. Yadav, R. K. Singh, Potential Effect of Acacia arabica on Peste des Petits Ruminants Virus Replication, Pharmaceutical Biology, 46:3, 171 – 179.
56. Van den Berghe, D.A., Vlietinck, A.J. and Van Hoof, L. (1986) Plant products as potential antiviral agents. Bulletin De L'Institut Pasteur 84, 101–107.
57. Wachsman, M.B., Castilla, V. and Coto, C.E. (1998) Inhibition of foot and mouth disease virus (FMDV) uncoating by a plant-derived peptide isolated from Melia azedarach L. leaves. Archives of Virology 143, 581–590.
58. Wagner, E.K. and Hewlett, M.J. (1999) Basic Virology. Malden, MA, USA: Blackwell Science. Wang, P. and Tumer, N.E. (1999) Pokeweed antiviral protein cleaves double-stranded supercoiled DNA using the same active site required to depurinate rRNA. Nucleic Acids Research 27, 1900–1905.
59. Wang, M.X., Cheng, H.W., Li, Y.J., Meng, L.M., Zhao, G. and Mai, K. (1995) Herbs of the genus Phyllanthus in the treatment of chronic hepatitis B: observations with three preparations from different geographic sites. Journal of Laboratory and Clinical Medicine 126, 350–352.
60. Webster.D. et al., Wang.X.,2006. immunostimulant property of heracleum maximum., j.Ethnopharmacol., 106(3), 360-363.
61. Xu, H.X., Zeng, F.Q., Wan, M. and Sim, K.Y. (1996) Anti-HIV triterpene acids from Geum japonicum. Journal of Natural Products 59, 643–645.
62. Yoshida, T., Ito, H., Hatano, T., Kurata, M., Nakanishi, T., Inada, A., Murata, H., Inatomi, Y., et al. (1996) New hydrolyzable tannins, shephagenins A and B, from Shepherdia argentea as HIV-1 reverse transcriptase inhibitors. Chemical and Pharmaceutical Bulletin (Tokyo) 44, 1436–1439.

Cite this article as:
Manoj Kumar, Anjay. Review on Antiviral and Immuno-Stimulatory Potential of Medicinal Plants. International Journal of Ayurveda and Pharma Research. 2016;4(9):12-19.
Source of support: Nil, Conflict of interest: None Declared

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