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Extracts and molecules from medicinal plants against herpes simplex viruses

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Abstract

Herpes simplex viruses (HSV-1 and -2) are important pathogens for humans, especially in the case of highly susceptible adults. Moreover, HSV-2 has been reported to be a high risk factor for HIV infection. Therefore, the discovery of novel anti-HSV drugs deserves great efforts. In this paper, we review anti-HSV substances from natural sources, including both extracts and pure compounds from herbal medicines, reported in studies from several laboratories. The role of traditional medicine for the development of anti-HSV compounds is also discussed. Interestingly, it was found that traditional medicines, like Ayurvedic, traditional Chinese (TCM), Chakma medicines, are good and potential sources for promising anti-HSV drugs. A second objective of this review is to discuss several anti-HSV compounds with respect to their structure–activity relationship (SAR). A large number of small molecules, like phenolics, polyphenols, terpenes (e.g., mono-, di-, tri-), flavonoids, sugar-containing compounds, were found to be promising anti-herpetic agents. Our major conclusion is that natural products from medicinal plant extracts are very important source of anti-HSV agents.

Keywords: Herpes simplex viruses; Herbal medicine; Acyclovir; Famciclovir; Acyclovir-resistance; Antiviral; Anti-HSV; Chakma Talika Chikitsa; Structure–activity relationship; Ethnomedicine; Traditional medicine

1. Introduction

1.1. Herpes simplex viruses (HSV) and related diseases

Herpes simplex viruses (both types, HSV-1 and -2) are pathogenic to humans.

After establishing latency, HSV can reactivate, causing frequent recurrent infections in some patients, while most people experience few recurrences. Among HSV-related pathologies, genital herpes is an important sexually transmitted disease (STD) commonly caused by HSV-2, with the exception of a minority of cases caused by HSV-1 (Johnson and Nahmias, 1989; Kalinyak et al., 1977; Corey et al., 1983; Yoosook et al., 1989). HSV-1 infections are very common and mostly affect adult people (Whitley and Kimberlin, 1998).

Abbreviations: ACV, acyclovir; ACV-R, acyclovir-resistant; ACV-S, acyclovir susceptible; ADMET, absorption distribution metabolism, excretion and toxicity; AIDS, acquired immune-deficiency syndrome; CC_{50}, median cytotoxic concentration; CPE, cytopathic effect; EC_{50}, median effective concentration; ED_{50}, median effective dose; EPTT, end-point titration technique; GC/MS, gas chromatography/mass spectrometry; GFS, galactofucan; HIV, human immunodeficiency virus; HSV, herpes simplex viruses; IC50, median inhibitory concentration; MIC, minimal inhibition concentration; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PB233′OG, prodelphinidin B-2 3,3′-di-O-gallate; PF, polysaccharide fraction; PPV, *Prunella vulgaris*; SAR, structure–activity relationship; SI, selectivity index; STD, sexually transmitted diseases; TCM, traditional Chinese medicine; TI, therapeutic index; TK, thymidine kinase; VSV, vesicular stomatitis virus; XTT, tetrazolium salt (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide)

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In genital herpes infections, the transmission of the virus by direct contact of recipient's mucous membranes or skin with infected sexual partner leads to the development of primary genital herpes (Hardin, 1996; Murray and Pizzorno, 1999). In case of genital herpes, HSV can reactivate to cause recurrent episodes as often as several times a year, sometimes for the remainder of a person's life (Tyring, 1998).

The primary symptoms of HSV infection include a prodromal "flu-like" syndrome, with fever, headache, malaise, diffuse myalgias, followed by local symptoms consisting of genital itching, tenderness, dysuria, lesions, painful papules over genital regions and ulceration (Hardin, 1996; Murray and Pizzorno, 1999).

The clinical manifestation of the disease exhibits different severity in normal and immuno-competent hosts; in addition, several patients always encounter recurrent attacks (Reeves et al., 1981; Whitley and Roizman, 1997). It should be pointed out that in immuno-compromised patients and neonates, HSV infections can cause serious systemic illnesses. In addition, HSV-2 infection may be a risk factor for the transmission of human immunodeficiency virus (HIV) (Severson and Tyring, 1999).

Furthermore, HSV is involved in several ocular diseases (Liesegang, 2001; Wilhelmus, 2000; Souza et al., 2003). For instance, herpetic stromal keratitis (HSK) is an immunopathological disease, which is one of the leading causes of blindness in the western world (Thomas and Rouse, 1997). Therefore, anti-HSV molecules could be of great relevance for infectious epithelial keratitis, neurotrophic keratopathy, stromal keratitis and endotheliitis (Holland and Schwartz, 1999).

Drug-resistant strains of HSV frequently develop following therapeutic treatment (Whitley and Kimberlin, 1998). Resistance to acyclovir and related nucleoside analogues can occur following mutation in either HSV thymidine kinase (TK) or DNA polymerase. Virus strains associated with clinical resistance are almost always defective in TK production (Weber and Cinatl, 1996). Therefore, new antiviral agents exhibiting different mechanisms of action are urgently needed. With respect to treatment, effective antiherpes drugs, such as acyclovir, ganciclovir, valaciclovir (L-valine ester of acyclovir), penciclovir, famciclovir (a prodrug of penciclovir (for structures see Fig. 1) with an improved oral bioavailability) and vidarabine, are available. Among these acyclovir is the most commonly used drug for treatment of HSV infections, followed by penciclovir/famciclovir. However, a serious problem of the use of acyclovir is drug resistance in treated patients. Moreover, it should be considered that these drugs are very expensive and several patients with frequent attacks may not be able to afford the cost of long-term treatment (Hammer and Inouye, 1997).

New prodrugs with increased bioavailability, such as valacyclovir and famciclovir, will perhaps prevent or delay the emergence of drug-resistant isolates in immunocompromised patients, since higher intracellular levels of the active compound can be achieved (Weber and Cinatl, 1996).

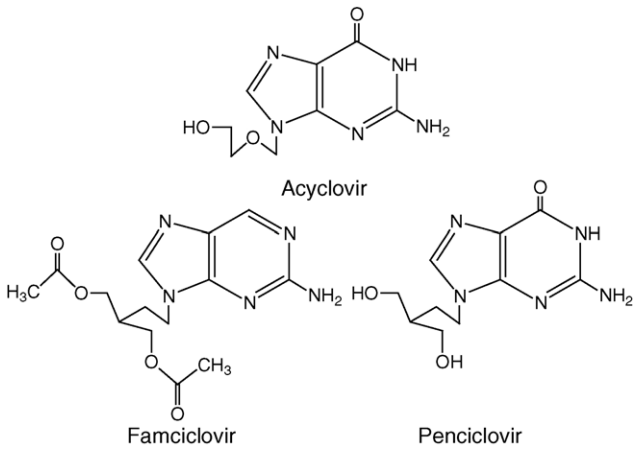


Fig. 1. Molecular structures of acyclovir, famciclovir and penciclovir.

The antiviral agents famciclovir, valacyclovir and acyclovir can be used to shorten the course and decrease the severity of these diseases and may suppress the virus itself, thereby preventing future outbreaks especially of the genital herpes (Tyring, 1998; Whitley and Kimberlin, 1998).

In relation to the involvement of HSV in the above mentioned diseases and to the problems related to drug resistance, large number of scientists is working to explore novel anti-HSV molecules.

1.2. Methods used for screening and/or studying anti-HSV agents

There are several in vitro and in vivo methods reported in the current literature to study the anti-herpetic activities of plant/herbal extracts or plant-derived molecules. Most commonly, researchers are using the cytopathic effect (CPE) on HSV-infected for preliminary studies and/or screening of large numbers of molecules/extracts.

Kurokawa et al. (1999) reported the anti-HSV activity of moronic and betulonic acids from the herbal extract of *Rhus javanica*. These molecules were derived from the ethylacetate soluble fraction of the herbal extract. First they performed a plaque reduction assay against the wild-type HSV-1 to get the median effective concentrations (EC_{50}) of the analyzed compounds and then they analyzed susceptibility of acyclovir–phosphonoacetic acid-resistant HSV-1, thymidine kinase-deficient HSV-1 and wild-type HSV-2. Finally, the compounds have been orally administered to mice cutaneously infected with HSV-1 to study their in vivo efficacy, including the survival time of the infected mice (Kurokawa et al., 1999).

A rapid and sensitive procedure to evaluate anti-HSV agents in vitro is based on spectrophotometrical assessment for viability of virus- and mock-infected cells via in situ reduction of a tetrazolium dye MTT. The method was proved to be as sensitive as plaque reduction assay. The system significantly simplifies the assay procedures, thus allowing the

evaluation of larger numbers of compounds for anti-HSV-1 activity (Takeuchi et al., 1991; Sudo et al., 1994).

Kira et al. (1995), reported the development of another highly sensitive HSV assay system by using a suspension cell line derived from human myeloma cells. The authors reported that these cells were sensitive for KOS (HSV-1 standard strain), A4D (HSV-1 ACV-resistant strain), Hangai (HSV-1 clinical isolate) and G (HSV-2 standard strain) strains, but not sensitive to the clinical isolates of HSV-2. Several known anti-HSV compounds including ACV, BVaraU, AraA, DHPG, PFA and DS, were studied using this system against KOS and G strains.

1.3. Treatment of HSV with natural products

Since a long time, medicinal plants have been used for the treatment of many infectious diseases, in most cases without a scientific background supporting their employment. On the contrary, there is, at present, increasing emphasis on determining the scientific evidence and rationale for the use of preparation from medicinal plants (Vermani and Garg, 2002). For instance, several research efforts are in progress to identify plants and their active components possessing activity against sexually transmitted pathogens, including HIV, with the objective of providing an effective approach for prevention of HIV transmission and treatment of AIDS (Vermani and Garg, 2002).

Accordingly, large number of synthetic and plant-derived anti-HSV drugs have been described in several studies (Martin, 1987; Andersen et al., 1991; Ferrea et al., 1993; Wood, 1999; Bourne et al., 1999; Ikeda et al., 2000). For instance, Vermani and Garg (2002), reviewed many plant-derived compounds investigated for their activity against sexually transmitted diseases, including those caused by HSV. They also discussed about alternative medicines, such as Unani, Chinese, Ayurvedic, naturopathy and homeopathy.

Another popular topical preparation (Pompei et al., 1979) for preventing and treating herpes outbreaks contains glycyrrhetinic acid, a triterpenoid component of *Glycyrrhiza glabra* (liquorice root). Glycyrrhizin has been found to improve the resistance of thermally injured mice to opportunistic infection of HSV-1, through induction of CD4+ T cells (Utsunomiya et al., 1995). In 2003, it was reported that a compound from broccoli inhibits HSV (Anon., 2003).

A final example has been reported by Bourne et al. who examined 19 plant-derived anti-microbial compounds in vitro by plaque reduction assay, in order to determine their activity against HSV-2. Compounds with an $ED_{50} \leq 7.0$ mg/ml were tested for efficacy in vivo. Four compounds, carrageenan lambda type IV, cineole, curcumin and eugenol, provided significant protection ($P < 0.05$) in a mouse model of intravaginal HSV-2 infection. Eugenol, which provided the greatest protection in mice, was also evaluated using a guinea pig model of genital HSV-2 infection, demonstrating significant protection.

Based on these results, it is clear that several plant-derived compounds warrant further evaluation as potential anti-HSV reagents (Bourne et al., 1999).

The objective of the present paper is to review the potential uses of natural products, especially derived from medicinal plants, for the treatment of infections caused by both types 1 and 2 HSV.

2. Natural anti-HSV products: from traditional knowledge to isolated pure compounds

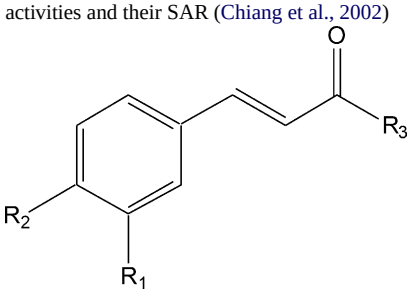
2.1. From the knowledge of folk or traditional medicines

We have recently reviewed interesting results from *Plantago major* L., a popular plant used in the traditional Chinese medicine (TCM) as well as other Asian traditional medicinal products, like Ayurveda (system of medicine used in the greater Indian sub-continent), Chakma Talika Chikitsa (a system of traditional medicine and treatments used by the largest tribe “Chakma” peoples in Chittagong Hill Tract of the Peoples Republic of Bangladesh) (Khan, 2002; Ather and Khan, 2005), for treating several diseases. In this respect, Chiang et al. (2002) examined the antiviral (on HSV-1, -2 and adenoviruses-3, -8, 11) activity of aqueous extracts and pure compounds of *P. major*. The results obtained showed that aqueous extract of *P. major* exhibit only a slight anti-herpes virus activity. By contrast, certain pure compounds belonging to the five different classes of chemicals found in extracts of this plant exhibited potent antiviral activity. Among them, caffeic acid exhibited the strongest activity against HSV-1 ($EC_{50} = 15.3$ μg/ml). The selectivity index (SI), determined by the ratio of CC_{50} (concentration causing 50% cellular cytotoxicity) to EC_{50} was 67.1. With respect to the activity against HSV-2, EC_{50} was found 87.3 μg/ml, SI was 118. It was concluded that the pure compounds isolated from *P. major* exhibiting anti-HSV activities are mainly derived from phenolic compounds, especially caffeic acid. The structures of the pure compounds, the activity spectrum and the structure–activity relationship (SAR) are shown in Table 1.

As shown in Table 1, the EC_{50} values of chlorogenic acid, caffeic acid and ferulic acid on HSV-2 were 20, 54 and 8000 μg/ml, respectively (Neyts et al., 1992; Bourne et al., 1999). These results definitely support the data by Chiang et al. (2002) indicating that consistent results showing anti-HSV activity of caffeic acid and some other related compounds have been reported by different laboratories.

Recently, Vijayan et al. (2004) studied 18 plants with ethnomedical background from different families, screened for the antiviral activity against HSV-1 on Vero cells using the cytopathic effect inhibition and virus yield reduction assays. Three extracts, namely *Hypericum mysorense*, *Hypericum hookerianum* and *Usnea complanta* exhibited significant antiviral activity at concentrations found to be non-toxic

Table 1
The structures of the pure compounds from *Plantago major*, their anti-HSV activities and their SAR (Chiang et al., 2002)



Compound	Functional groups			Anti-HSV activities (EC_{50}, μg/ml)	
	R_1	R_2	R_3	HSV-1	HSV-2
Caffeic acid	–OH	–OH	–OH	15.3	87.3
Chlorogenic acid	–OH	–OH	X*	47.6	86.5
Ferulic acid	–OH	–OCH3	–OH	>100	>100
p-Coumaric acid	–OH	–H	–OH	>200	32.8
Acyclovir (as standard)	–	–	–	1.9	1.5

Note: X*, 1,3,4,5-tetrahydroxycyclohexane carboxylic acid.

to the cells. The extracts from *Melia dubia*, *Cryptostegia grandiflora* and the essential oil of *Rosmarinus officinalis* exhibited partial activity only at higher concentrations.

Several medicinal plants of North and South America have been reported to have promising anti-HSV activities. For instance, selected plants having a history of use in Polynesian traditional medicine for the treatment of infectious diseases were investigated by Locher et al. (1995) for antiviral, anti-fungal and anti-bacterial activity in vitro. Extracts from *Scaevola sericea*, *Psychotria hawaiiensis*, *Pipturus albidus* and *Eugenia malaccensis* showed selective antiviral activity against HSV-1 and -2.

In another report by Kott et al. (1999), the ethnopharmacological screening of selected medicinal plants used in Argentina for the treatment of infectious diseases was undertaken, including analysis of aqueous extracts of five species, assayed in vitro to detect antiviral activity against HSV-1. *Polygonum punctatum*, *Lithraea molleoides*, *Sebastiania brasiliensis* and *Sebastiania klotzschiana* but not *Myrcianthes cisplatensis* showed in vitro anti-herpetic activity with ED_{50} ranging from 39 to 169 μg/ml. The differences between their maximal non-cytotoxic concentration and their antiviral activity values were high enough to justify further analysis.

In a study using a large number of plant extracts, 47 extracts of 10 species of the genus *Euphorbia* (Euphorbiaceae), used by Colombian traditional healers for the treatment of ulcers, cancers, tumors, warts and other diseases, were tested in vitro and reported by Betancur-Galvis et al. as potential anti-tumor (anti-proliferative and cytotoxic) and anti-herpetic natural product (Betancur-Galvis et al., 2002). To evaluate the ability of the extracts to inhibit the lytic activity of HSV-2 and the reduction of viability of infected or uninfected cell cultures, the end-point titration technique (EPTT) and the MTT colorimetric assay were used. Five of the 47 extracts (11%) representing 3 out of 10 *Euphorbia* species (30%) exhibited anti-herpetic action; the highest activity was found in the leaf/stem water–methanol extracts from *E. cotinifolia* and *E. tirucalli*, with a TI > 7.1.

Furthermore, hydromethanolic extracts prepared from 54 Brazilian medicinal plants used in folk medicine to treat infections were screened for antiviral properties against five different viruses (HSV-1, HSV-2, poliovirus type 2, adenovirus type 2 and VSV) (Simoes et al., 1999a). Fifty-two percent of the plant extracts exhibited antiviral activity against one or more tested viruses. Among these, 42.6% showed activity against HSV-1, 42.6% against HSV-2. *Trixis praestans* (Vell.) Cabr. and *Cunila spicata* Benth. extracts were further characterized for their antiviral activity (Simoes et al., 1999a).

As a final example, several plants used in Nepalese traditional medicine were found to display antiviral activity. Methanolic and methanolic-aqueous extracts derived from 23 species were assayed in two in vitro viral systems, influenza virus/MDCK cells and HSV/Vero cells. The *Holoptelia integrifolia* and *N. indicum* exhibited considerable antiviral activity against HSV. None of these extracts showed cytotoxic effects. Additionally, for *B. ligulata* and *H. integrifolia* partial protease inhibitory activity was estimated (Rajbhandari et al., 2001). Table 2 summarizes the results of this screening.

2.2. Unfractionated plant extracts showing promising anti-herpetic activities

A very high number of unfractionated extracts from medicinal plants have been shown to retain anti-HSV activity. Furthermore, 20 medicinal plant extracts from Thailand have been evaluated for their biological activity against HSV-1 by Lipipun et al. (2003). Eleven of them inhibited more than 50% plaque formation of HSV-1 when was added 100 μg/ml in plaque reduction assay. Among these plant extract, those obtained from *Aglaia odorata*, *Moringa oleifera* and *Ventilago denticulate* were also effective against thymidine kinase

Table 2
Nepalese medicinal plants used to prepare anti-HSV-1 extracts (modified from Rajbhandari et al., 2001)

Plant extracts	Family	Solvents used for extraction	IC_{50} (μg/ml)
Bergenia ligulata	Saxifragaceae	Methanol–water	70
Bergenia ligulata	Saxifragaceae	Methanol	56
Bombax cieba	Bombaceae	Methanol–water	62
Carea arborea	Lecythidaceae	Methanol	10
Coccinia cordifolia	Cucurbitaceae	Methanol	67
Dypsacus mitis	Dipsaceaceae	Methanol–water	82
Holoptelia integrifolia	Ulmaceae	Methanol	10
Nerium indicum	Apocynaceae	Methanol	10
Rhododendron anthopogon	Ericaceae	Methanol–water	38
Rhododendron anthopogon	Ericaceae	Methanol	70
Salvia coccinia	Labiateae	Methanol–water	62
Salvia coccinia	Labiateae	Methanol	98

Table 3
Anti-HSV assay of some plant extracts from Thailand, employing plaque reduction assay and ACV (modified from Lipipun et al., 2003)

Plant extracts	Family	Plaques percent of control (100 μg/ml)	EC_{50} (μg/ml)
Aglaia odorata	Meliaceae	0^{Δ}	9.5 ± 0.7
Cerbera odollam	Apocynaceae	0^{Δ}	0.4 ± 0.1
Moringa oleifera	Moringaceae	49.5^{Δ}	100.0 ± 5.3
Ventilago denticulata	Rhamnaceae	0^{Δ}	46.3 ± 1.5
Willughbeia edulis	Apocynaceae	100	–
ACV	–	–	0.2 ± 0.1

Note: $^{\Delta}$Plant extracts that reduced plaque formation of HSV-1 more than 50%.

(TK)-deficient HSV-1 and phosphonoacetate-resistant HSV-1 strains (Lipipun et al., 2003). The activity of some of these plant extracts is tabulated in Table 3 and compound to that exhibited in similar experiments by acyclovir.

A similar study employing three (*Hemidesmus indicus*, *Paederia foetida* and *Shorea robusta*) medicinal plants from Bangladesh, was recently reported by Khan et al. (2005) against 38 strains of HSV: 18 strains (7 HSV-1 and 11 HSV-2) were resistant to acyclovir (ACV-R) and 20 strains (10 HSV-1 and 10 HSV-2) were susceptible to ACV (ACV-S). The IC_{50} of each extract for the seven ACV-R strains of HSV-1 were 200 μg/ml for *H. indicus*, 400 μg/ml for *P. foetida* and 10 μg/ml for *S. robusta*. The IC_{50} of the extracts tested against the 11 ACV-R strains of HSV-2 were 200 μg/ml for *H. indicus*, 200 μg/ml for *P. foetida* and 5.0 μg/ml for *S. robusta*. When ACV-S strains of HSV-1 were employed the IC_{50} were 400, 800 and 20 μg/ml, for *H. indicus*, *P. foetida* and *S. robusta*, respectively, and with HSV-2 ACV-S strains, the IC_{50} values were 100, 200 and 10 μg/ml for *H. indicus*, *P. foetida* and *S. robusta*, respectively. The cytotoxicity of the three herbal extracts was 8000 μg/ml for *H. indicus*; 8000 μg/ml for *P. foetida* and 2000 μg/ml for *S. robusta*. Although the active component(s) in the extracts responsible for anti-HSV activity has not been identified, all extracts displayed good antiviral activity against both ACV-R and ACV-S strains of HSV-1 and HSV-2, and their mechanism of action was found to be a strong inhibition of the binding of HSV to cellular receptors (Khan et al., 2005).

Similarly, in a recent paper, a decoction from *Astragalus membranaceus* has been investigated against HSV-1, using 2BS cells infected with HSV-1 amounts causing cytopathic effects. The IC_{50} and the minimal inhibition concentration (MIC) of *Astragalus membranaceus* extracts were 0.98 and 1.95 mg/ml, respectively, with a therapeutic index (TI) of 128. From this study, it was concluded that *A. membranaceus* extracts exhibit HSV-1-inhibiting efficacy and low cytotoxicity (Sun and Yang, 2004).

The extract from the red marine alga *Ceramium rubrum* (Huds.) Ag., from the Bulgarian Black Sea seacoast, inhibited the reproduction of HSV types 1 and 2 in cell cultures. The preparation exhibited a strong HSV-inactivating activity (Serkedjieva, 2004).

Methanol extracts of 36 medicinal plants from La Reunion Island were evaluated against HSV-1 and five of them were reported to be active against HSV-1 (Fortin et al., 2002). Among the 36 plants tested, *Obetia ficifolia* and *Erythroxylum laurifolium* appeared to be the most active extracts against HSV-1. For *O. ficifolia* this effect might be due to the high content of triterpenes, whereas for *E. laurifolium* the activity might be related to the presence of condensed tannins. Therefore, this effect would be probably non-selective. *Olax psittacorum* and *Psiadia dentata* showed the best inhibition activity against poliovirus. Among the nine plants exhibiting antiviral activity, four (*Citrus hystrix*, *E. laurifolium*, *O. ficifolia* and *Senecio ambailla*) are used in folk medicine. Extracts from these four plants inhibited herpes virus, even if their therapeutic use was not always recommended for the viral disease.

Several medicinal plants exhibiting anti-HSV activity were studied in virtue of their previously described biological activities. This is the case of *Helichrysum litoreum* Guss, a Campanian medicinal plant reported to have anti-bacterial properties. Extracts from this plant were studied by Guarino and Sciarrillo (2003) for their antiviral activities against HSV-1 in vitro. The crude aqueous extract from leaves of *H. litoreum* exhibited at 1.35 mg/ml significant antiviral activity against HSV-1 in human lung fibroblast and were demonstrated to exhibit absence of CPE.

In addition, screening of medicinal plants already used in ethnopharmacology has been demonstrated to be a productive approach. An example is the herbal remedies that play a fundamental role in traditional medicine in rural areas of Colombia, where they are often the therapeutic treatment of choice. Lopez et al. (2001) reported strong antiviral and antimicrobial activities of methanolic extracts of 24 plants used in the treatment of skin infections in 4 different regions of Colombia. Thirteen extracts displayed activity against HSV-1. The antiviral activity was indicated by a total inhibition of viral CPE at a non-cytotoxic concentration of the extract. The most potent extract was obtained from *Byrsonima verbascifolia* (L.) HBK, which showed anti-HSV-1 activity at a concentration as low as 2.5 μg/ml (Lopez et al., 2001) (see Table 4 for a partial list of the characterized anti-HSV extracts).

2.3. Fractionated plant extracts showing anti-HSV activities

Starting from observations using unfractionated plant extracts exhibiting anti-HSV activity, several laboratories tried to identify bioactive fractions. For instance, Chiu et al. (2004) reported a study on the polysaccharide fraction (PF) derived from *Prunella vulgaris* (PPV), a perennial plant commonly found in China. In their report, the expression of HSV antigen in Vero cells was analyzed by flow-cytometry and found to be time dependently increased in infected cells. The polysaccharide fraction of PPV reduced its expression, being the EC_{50} of HSV-1 and -2 antigens 20.6 and 20.1 μg/ml,

Table 4
Potential list of medicinal plants used in the Colombian traditional medicine active against HSV-1 (modified from Lopez et al., 2001).

Family	Species	Parts	MIC$^{\Delta}$ (μg/ml)
Clusiaceae	*Vismia macrophylla*	Resin	5.5
	Symphonia globulifera	Bark	25
Lecythidaceae	*Eschweilera rufifolia*	Bark	8
Malphigiaceae	*Byrsonima verbascifolia*	Root bark	6.5
	Byrsonima verbascifolia	Leaves	2.5
Menispermaceae	*Iryanthera megistophylla*	Bark	10
Myristicaceae	*Virola multinervia*	Resin	11.5
	Virola multinervia	Bark	17
Myrtaceae	*Myrteola nummulari*	Aerial parts	10.5
Polygonaceae	*Polygonum punctatum*	Aerial parts	20
Pteridophyta	*Adiantum latifolium*	Aerial parts	11.5
Rhamnaceae	*Ampelozizyphus amazonicus*	Leaves	22
Rubiaceae	*Duroia hirsuta*	Leaves	10.5

Note: $^{\Delta}$Minimum concentration causing complete inhibition (MIC) of HSV.

respectively. Furthermore, Chiu et al. claimed that the novelty of PPV was its ability to reduce the antigen expression of acyclovir-resistant strain of HSV-1. In fact, using acyclovir-resistant strains, after incubations with 25–100 μg/ml of PPV, the HSV antigen-positive cells were reduced by 24.8–92.6%, strongly suggesting that PF of PPV exhibits an anti-HSV mechanism of action different from that of acyclovir. In conclusion, their results demonstrated that the PF of PPV is effective against both HSV-1 and -2 infections, and flow-cytometry offers a quantitative and highly reproducible anti-HSV drug-susceptibility assay.

2.4. Pure compounds showing anti-HSV activities

As far as identification of bioactive anti-HSV molecules and structure–activity relationship studies, several reports have been recently published. Describing an impermeably high number of pure compounds from natural products exhibiting promising anti-HSV activities. Some of these are discussed in brief in this chapter according to their chemical classes.

2.5. Polyphenolics

Cheng et al. (2003) studied the in vitro antiviral properties of prodelphinidin B-2 3,3′-di-*O*-gallate (PB233′OG), isolated from the bark of *Myrica rubra* (Myricaceae). Their results demonstrated that PB233′OG exhibits anti-HSV-2 activity with IC_{50} values of 5.3 and 0.4 μM for XTT and plaque reduction assays, respectively. The IC_{50} value increased with increasing multiplicity of infection (MOI). Interestingly, PB233′OG did not show a cellular cytotoxic effect at concentrations exhibiting antiviral activity. As far as mechanism of action, their studies demonstrated that PB233′OG on one hand inhibits HSV-2 attachment to Vero cells, interfering with the penetration of HSV-2 into cells, on the other hand affects the late stage(s) of HSV-2 infection cycle, reducing the viral infectivity at high viral loading. It was concluded that PB233′OG exhibits several mechanisms of action leading to its anti-HSV-2 effects (Cheng et al., 2003).

Buckwold et al. recently reported crude hop extracts and purified hop components representing the major chemical class of hop compounds display antiviral activity against HSV-1 and -2 and some other important pathogenic viruses. A xanthohumol-enriched hop extract displayed a weak to moderate antiviral activity against HSV-2 ($TI \geq 5.3$), and HSV-1 ($TI \geq 1.9$) with IC_{50} values in the low μg/ml range. No antiviral activity was detected using beta-acids or a hop oil extract. Ultra-pure preparations were used to show that xanthohumol accounted for the antiviral activity observed in the xanthohumol-enriched hop extract against HSV-1 and -2. Xanthohumol was found to be an antiviral agent against these viruses more potent than the isomer *iso*-xanthohumol. Buckwold et al. concluded that these hop constituents might serve as interesting lead compounds from which more active anti–HSV antiviral agents could be synthesized and tested (Buckwold et al., 2004).

The antiviral and antioxidant activity of some fractions and of a series of flavonoids and proanthocyanidins obtained from *Crataegus sinaica* (Rosaceae) was evaluated by Shahat et al. (2002). *O*-Glycosidic flavonoids and oligomeric proanthocyanidins exhibited significant inhibitory activity against HSV-1, which was shown to be due to an extracellular mechanism for procyanidin C-1.

A very interesting example of SAR is related to the study of a series of dimeric procyanidins (compounds 1–9 of Fig. 2) and some related polyphenols (compounds 10–15 of Fig. 3), chosen as model compounds in a comparative investigation for several biological activities, including anti-HSV effects. In general, more pronounced anti-HSV activities were seen with epicatechin-containing dimers, while the presence of ortho-trihydroxyl groups in the B-ring was important in compounds exhibiting anti-HSV activity. The double interflavan linkages lead to a significant increase of the anti-HSV effects (De Bruyne et al., 1999).

An interesting example of identified bioactive product is related to propolis (bee-glue), a natural resinous material assembled by the honey bees on the buds of various trees, such as poplar (*Populus* spp.), birch (*Betula alba*), beech (*Fagus sylvatica*), horse-chestnut (*Aesculus hippocastanurn*), alder (*Ahus glutinosa*) and various conifers. The in vitro activity against HSV-1 of the major flavonoids identified in propolis was investigated by Amoros et al. (1992). As regards the action of flavonoids against HSV, quercetin, procyanidin, and pelargonidin were found to be virucidal (Mucsi et al., 1977), whereas luteolin was inactive. The direct inactivation of HSV by quercetin, catechin and hesperitin has been verified (Kaul et al., 1985). Flavonols were found to be more active than flavones, the activity decreasing in the reverse order of the numbers of their hydroxyl substitution, i.e. galangin > kaempferol > quercetin. The efficacy against

HSV-1 of binary flavone–flavonol combinations has been also investigated. The synergy demonstrated by all combinations could explain why propolis is more active than its individual compounds (Amoros et al., 1992). Quercetin was found to be effective when the time of incubation was about 24 h, i.e. when the assays were performed after one multiplication cycle. It was considered ineffective when the incubation time was 3 days (Amoros et al., 1992).

Fig. 3 shows the molecular structures of flavone, flavanone and some flavonoids.

2.6. Glycosides

In a recently published paper, Nohara (2004) reviewed his extensive investigation of the isoprenoidal glycosides in Solanaceae and Leguminosae folk medicines, to verify their anti-HSV activities. Their structure–activity relationships have been discussed.

2.7. Terpenes:monoterpenes

Fig. 4 shows some monoterpenes, like cineole (eucalyptol), isoborneol and borneol, etc., reported to have potent anti-HSV activities.

One report discussing the anti-HSV activity of monoterpenes was based on the study of essential oils from the leaves of the Egyptian plants *M. ericifolia*, *M. leucadendron*, *M. armillaris* and *M. styphelioides* were studied in Vero cells by a plaque reduction assay. The volatile oil of *M. armillaris* was more effective as virucidal (up to 99%) than that of *M. leucadendron* (92%) and *M. ericifolia* (91.5%). The essential oils were isolated by a hydrodistillation method and analysed by a gas chromatography/mass spectrometry (GC/MS) technique. The essential oil of *M. ericifolia* contained methyleugenol (96.84%) as a major constituent, whereas *M. leucadendron* was rich in 1,8-cineole (64.30%), *M. armillaris* was also rich in 1,8-cineole (33.93%) followed by terpinen-4-ol (18.79%),

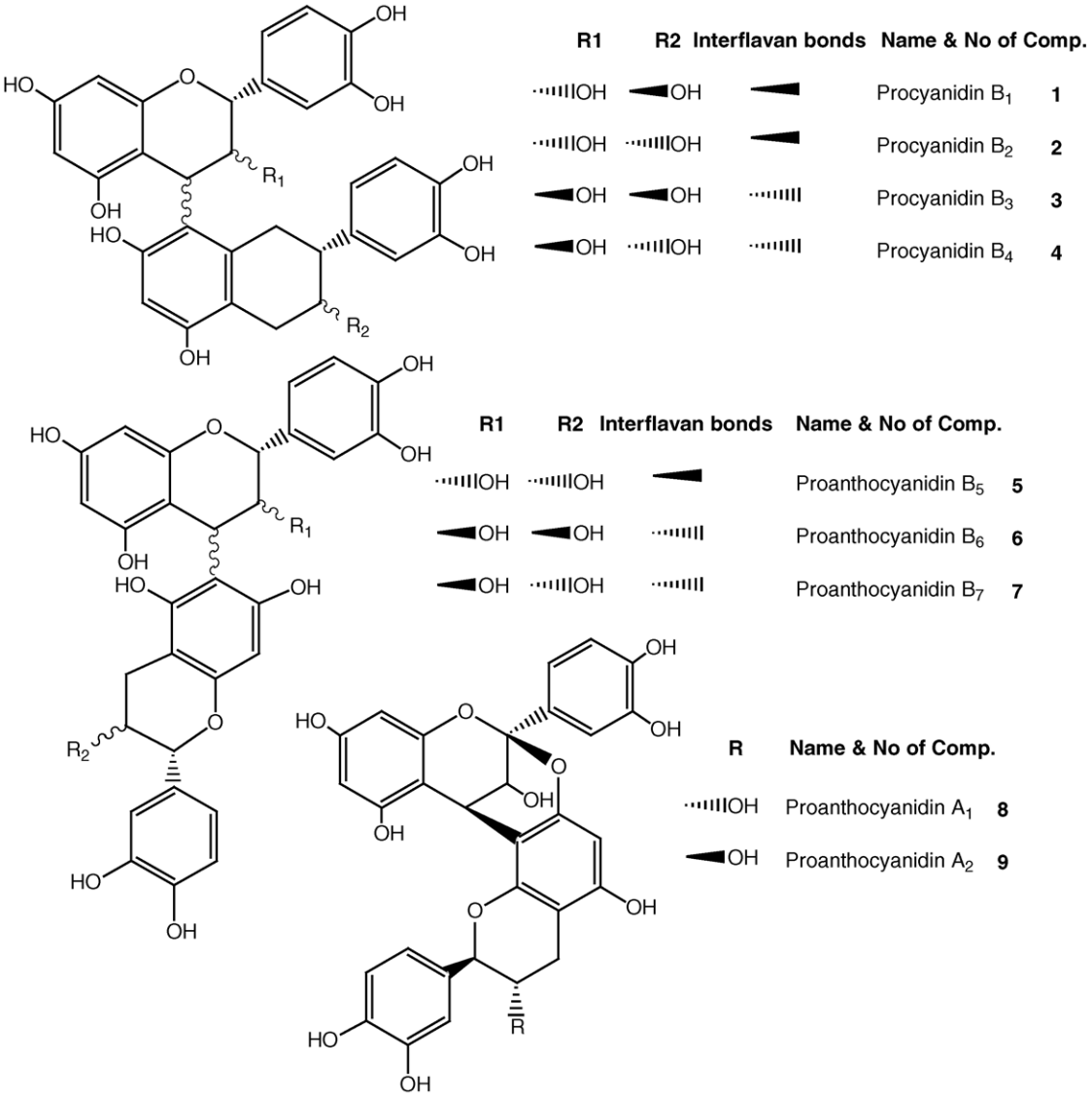


Fig. 2. Molecular structure of the anthocyanidine and catechins (from De Bruyne et al., 1999).

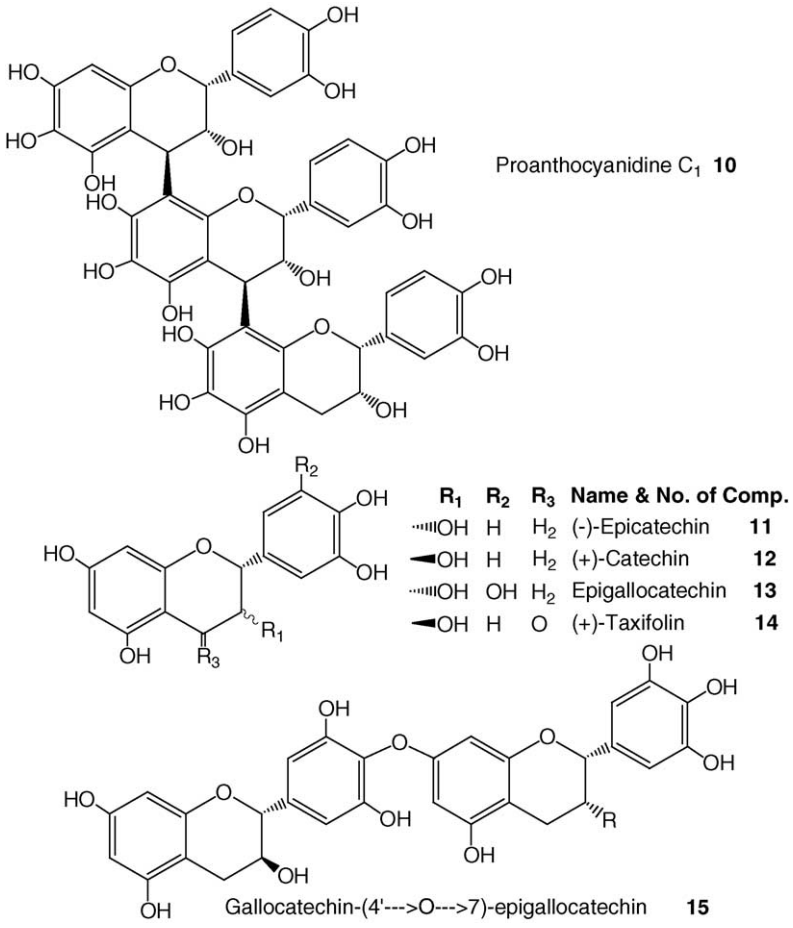


Fig. 2. (*Continued*).

whereas *M. styphelioides* was rich in caryophyllene oxide (43.78%) and (−) spathulenol (9.65%) (Farag et al., 2004).

On the other hand, Armaka et al. reported that isoborneol (for structure see Fig. 4), a monoterpene component of several plant essential oils, exhibited interesting anti-HSV-1 activity. First, it inactivated HSV-1 very effectively within 30 min of exposure, and second, at a concentration of 0.06% completely inhibited viral replication, without affecting viral adsorption. The molecule did not exhibit significant cytotoxicity when used at concentrations ranging between 0.016 and 0.08%, when tested against human and monkey cell lines. It specifically inhibited glycosylation of viral polypeptides,

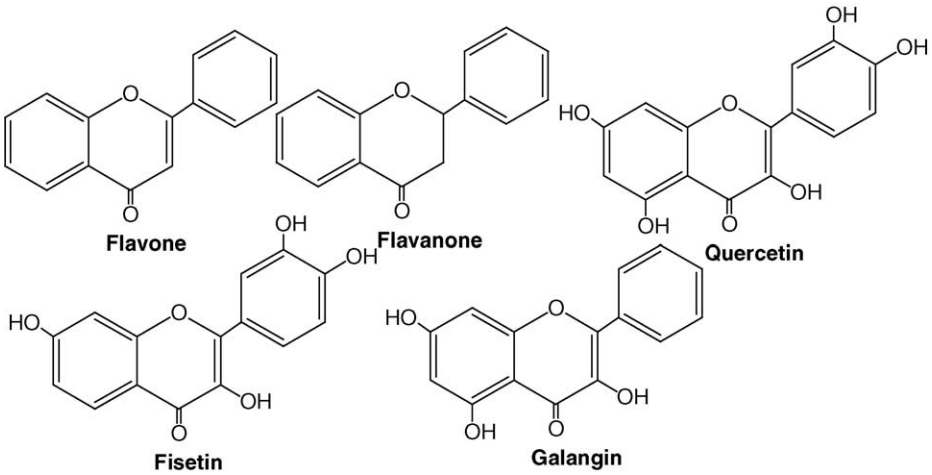


Fig. 3. Structures of some flavonoids exhibiting anti-HSV activity.

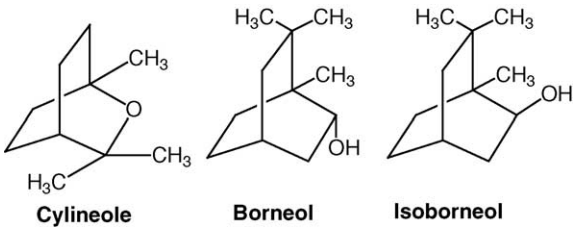


Fig. 4. Structures of some monoterpenes found in plant extracts active against HSV.

based on the experimental evidence that the mature fully glycosylated forms of two viral glycoproteins, gB and gD, were not detected when the virus was replicated in the presence of isoborneol. Interestingly, no major changes were observed in the glycosylation pattern of cellular polypeptides, when untreated and isoborneol-treated Vero cells were compared. Furthermore, isoborneol did not affect the glycosylation of gB produced from a copy of the gB gene present in the cellular genome. Finally, other monoterpenes, such as 1,8-cineole and borneol (a stereoisomer of isoborneol), did not inhibit HSV-1 glycosylation (Armaka et al., 1999). Taken together, these data allow to consider isoborneol as a very interesting compound for inhibiting HSV life cycle.

2.8. Terpenes:diterpenes

In another study, Cheng et al. recently reported that putranjivain A, isolated from the whole plant *Euphorbia jolkini* Bioss (Euphorbiaceae), and studied on Vero cells infected with the HSV-2 strain 196, retains in vitro antiviral properties against HSV-2 (Cheng et al., 2004), the IC_{50} value was found to be 7.9 μM using the XTT assay. Using the plaque reduction assay, the IC_{50} and IC_{90} values were 6.3 and 14.5 μM, respectively. Putranjivain A exhibited no inhibitory effect on cell proliferation at concentrations leading to anti-HSV-2 activity. The CC_{50} value was 80.3 μM, and the SI for XTT and plaque reduction assays was 10.2 and 12.7, respectively. When tested for virucidal activity, putranjivain A significantly reduced viral infectivity at concentrations of 75 and 100 μM, but not at 50 μM or below. The results of time-of-addition studies suggested that putranjivain A affected the late stage of HSV-2 replication at 25 μM. Interestingly, putranjivain A also exhibited inhibition of viral attachment and cell penetration. Combination of putranjivain A and acyclovir produced no increased anti-HSV activity. From the study by Cheng et al. (2004), it can be concluded that the compound putranjivain A exhibits anti-HSV-2 activity, inhibiting viral attachment and penetration, and also interfering with the late stage of viral replication (Cheng et al., 2004).

2.9. Terpenes: triterpenes

From the medicinal plant *R. javanica*, which exhibits oral therapeutic anti-HSV activity in mice, Kurokawa et al. (1999) purified two major anti-HSV compounds from the ethyl acetate fraction, moronic acid and betulonic acid and examined their anti-HSV activity in vitro and in vivo. Moronic acid was found to be the major anti-HSV compound. The EC_{50} of moronic acid and betulonic acid (see Fig. 5) for wild-type HSV-1 were 3.9 and 2.6 μg/ml, respectively. The TI of moronic acid (10.3–16.3) was larger than that of betulonic acid (6.2). Susceptibility of ACV–phosphonoacetic acid-resistant HSV-1, thymidine kinase (TK)-deficient HSV-1 and wild-type HSV type 2 to moronic acid was similar to that of the wild-type HSV-1. When this compound was administered orally three times daily to mice infected cutaneously with HSV-1, it significantly retarded the development of skin lesions and/or prolonged the mean survival times of infected mice without toxicity compared with control mice. Moronic acid suppressed virus yields to the brain more efficiently than those to the skin. This was consistent with prolongation of mean survival times. Thus, moronic acid was isolated and characterized as a major anti-HSV compound from herbal extracts of *R. javanica*, with a mechanism of action different from that of ACV.

In another study, Alche et al. (2002) reported that isolated meliacine (MA), an antiviral principle from the leaves of *Melia azedarach* L., exhibits potent antiviral activity against HSV-1, by inhibiting specific infected-cell polypeptides (ICPs) produced late in infection. Some of these properties are involved in DNA synthesis and in the assembly

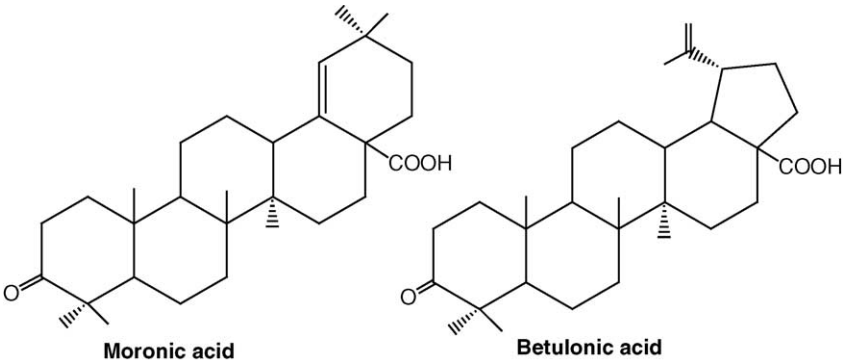


Fig. 5. Molecular structures of some triterpenes found to be active against HSV (from Kurokawa et al., 1999).

of nucleocapsids. The same research group provided additional evidence to elucidate the mechanism of action of MA against HSV-1. Time-of-addition experiments confirmed that MA affects a late event in the life cycle of HSV-1. They also showed that MA diminished the synthesis of viral DNA and inhibited the spread of infectious viral particles when HSV-1 expressing β-galactosidase activity was used as a detection system. In addition, the lack of a protein with an apparent MW of 55 KDa was detected in MA-treated cell extracts. Ultrastructural analysis of infected cells showed that, after MA treatment, a large number of unenveloped nucleocapsids accumulated in the cytoplasm and a minor proportion of mature virus was found in cytoplasmic vesicles. These findings suggested that MA exerts the antiviral action on both synthesis of viral DNA and maturation and progress of HSV-1 during infection of Vero cells.

Finally, we would like to mention the studies by Simoes et al. (1999a,b) and Madureira et al. (2003).

Simoes et al., reported and explained the possible mechanism of antiviral activity of triterpenoid saponins (Simoes et al., 1999b), naturally occurring sugar conjugates of triterpenes, possessing several biological activities, including antiviral action. The triterpenoid saponin, isolated from a Brazilian plant, represents the oleanane group and inhibited HSV type 1 DNA synthesis. The triterpenoid saponin, isolated from a Chinese plant, represents the ursane group and seemed to inhibit viral capsid protein synthesis of HSV-1. They did not show evidence of cytotoxicity under antiviral test conditions (Simoes et al., 1999b).

Madureira et al. (2003) reported the phytochemical reinvestigation of the whole plant of *Euphorbia segetalis*, yielding five tetracyclic triterpenes: 3β-hydroxy-cycloart-25-en-24-one, cycloart-25-ene-3β,24-diol, cycloart-23-ene-3β,25-diol, lanosta-7,9(11),24-trien-3β-ol and lanosta-7,9(11),24(31)-trien-3β-ol. The β-acetoxy-cycloart-25-en-24-one and glutinol, lupenone, dammaranodienol, cycloartenol acetate, 24-methylenecycloartanol acetate and β-sitosterol, isolated previously, and also have been evaluated for their antiviral activities against HSV. The conclusion of these studies demonstrated that lupenone exhibited strong viral plaque inhibitory effect against HSV-1 and -2 (Madureira et al., 2003).

2.10. Polysaccharide

As far as studies on isolated compounds, very recently Thompson and Dragar (2004), reported that galactofucan (GFS), a sulfated polysaccharide known as the major component of an aqueous extract from the seaweed *Undaria pinnatifida*, exhibits anti-HSV activities. The authors evaluated GFS for antiviral activity against 32 clinical strains of HSV: 14 strains of HSV-1 and 18 strains of HSV-2. Twelve strains (4 HSV-1 and 8 HSV-2) were resistant to acyclovir (ACV-R) and 20 strains (10 HSV-1 and 10 HSV-2) were susceptible to ACV (ACV-S). The median IC_{50} values of GFS were 32 and 0.5 μg/ml against HSV-1 and -2, respectively,

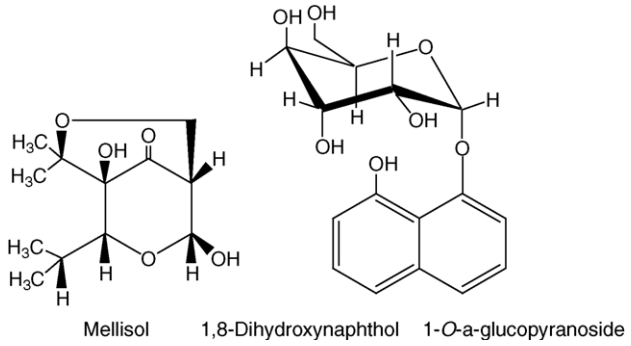


Fig. 6. Structures of a unique polyketide mellisol with 1,8-dihydroxynaphthol 1-*O*-α-glucopyranoside, which exhibited potent anti-HSV-1 activity.

demonstrating that this compound is significantly more active against clinical strains of HSV-2 than HSV-1 ($P < 0.001$). The mode of action of GFS was shown to be inhibition of viral binding and entry into the host cell. The cytotoxicity, evaluated by the neutral red dye uptake assay was >4.0 mg/ml, indicating that GFS is non-toxic in this assay.

2.11. Polyketides

Very recently Pittayakhajonwut et al., 2005 have reported a structurally unique polyketide mellisol with 1,8-dihydroxynaphthol 1-*O*-α-glucopyranoside (for structures see Fig. 6) showing potent anti-HSV-1 activity, having IC_{50} values of 10.50 and 8.40 μg/ml, respectively. These compounds were isolated from the fungus *Xylaria mellisii* (BCC 1005).

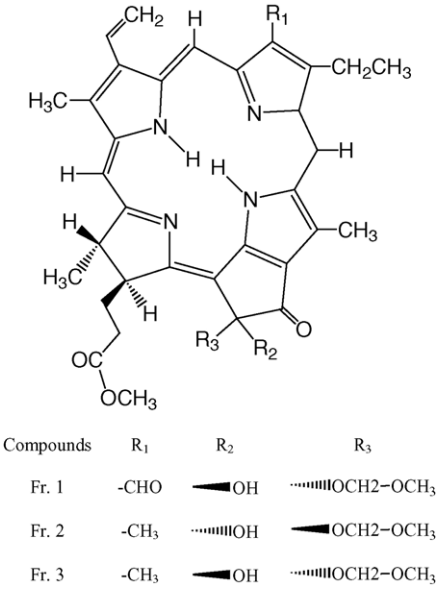


Fig. 7. Structures of some novel pheophorbide-like anti-HSV molecules isolated from marine green alga *Dunaliella primolecta* (molecular structures extracted from Ohta et al., 1998).

2.12. Pheophorbides

Ohta et al. (1998) investigated the cytopathic effect (CPE) upon Vero cells of HSV-1 on 106 microalgae, among them the methanolic extracts of the *Dunaliella bioculata* C-523, *D. primolecta* C-525, *Lyngbya* sp. M-9 and *Lyngbya aerugineo-coerulea* M-12 inhibited the CPE and the *D. primolecta*, exhibited the highest anti-HSV-1 activity at 10 μg/ml of extract from this alga completely inhibited the CPE. This activity was similar to that of acyclovir at the same concentration. Using different chromatographic techniques they have purified three pheophorbide-like compounds (for structure see Fig. 7), found to completely inhibit the CPE at 5 μg/ml.

3. Concluding remarks

In this short review, we presented results concerning the antiviral activity of several plant extracts and some pure compound isolated from natural resources for the treatment of HSV-1 and -2.

Our conclusion is that extracts from plants employed in ethnomedicine can exhibit antiviral activity against HSV, both the types 1 and 2. Accordingly, medicinal plants can be a source for the isolation of pure compounds acting against HSV-1 and -2. Despite the fact that the amount of information on anti-HSV plant extracts is very relevant, not all the bioactive anti-HSV molecules responsible for the activity of plant extracts have been identified, isolated, synthesized and tested.

This is a crucial point, and it is highly advisable that all the most promising plant extracts should undergo further analysis and purification steps in order to identify the active principle(s) and clarify the chemical nature and mechanism(s) of action of the potential anti-HSV molecule(s).

On the other hand, several molecules have been already tested for anti-HSV activities in vitro and/or in vivo; the most potent molecule(s) of these should undergo preclinical as well as toxicity evaluations. Synthetic approach(es) should also be developed for the production of larger amounts of bioactive molecules and their analogues for all the required preclinical studies as well as for clinical studies on healthy subjects and HSV-infected patients.

Finally, gene expression profile studies employing microarray technology could help to identify molecular targets of the biological activity of anti-HSV molecules from natural products, allowing to move to gene-based drugs to be used in anti-HSV therapy.

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