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Efficacy of an aqueous *Pelargonium sidoides* extract against herpesvirus

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Abstract

The compounds of an aqueous root extract of the African medicinal plant *Pelargonium sidoides* were analysed by LC–MS spectroscopy and the antiviral effect of this extract against herpes simplex virus was examined in cell culture. Besides predominant coumarins, simple phenolic structures as well as flavonoid and catechin derivatives were identified as major constituents in the Pelargonium extract. The inhibitory activity of this extract against herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) was tested *in vitro* on RC-37 cells using a plaque reduction assay and exhibited high antiviral activity against both herpesviruses in viral suspension tests. The 50% inhibitory concentration (IC_{50}) of the aqueous *Pelargonium sidoides* extract for herpes simplex virus plaque formation was determined at 0.00006% and 0.000005% for HSV-1 and HSV-2, respectively. At maximum noncytotoxic concentrations of the extract, plaque formation was significantly reduced by more than 99.9% for HSV-1 and HSV-2 and a clear concentration-dependent antiviral activity against HSV could be demonstrated for this extract. In order to determine the mode of antiviral action, the extract was added at different times to the cells or viruses during the infection cycle. Both herpesviruses were significantly inhibited when pretreated with the plant extract or when the extract was added during the adsorption phase, whereas acyclovir demonstrated antiviral activity only intracellularly during replication of HSV. These results indicate that *P. sidoides* extract affected the virus before penetration into the host cell and reveals a different mode of action when compared to the classical drug acyclovir. Hence this extract is capable of exerting an antiviral effect on herpes simplex virus and might be suitable for topical therapeutic use as antiviral drug both in labial and genital herpes infection.

Keywords: *Pelargonium sidoides*; Antiviral activity; Herpes simplex virus; Aqueous extract

Introduction

The roots of the South African species *Pelargonium sidoides* DC (*Geraniaceae*) are used in traditional medicine as an antidiarrhoic and in general for the treatment of colds and lung infection including tuberculosis (Matthys et al., 2003; Bladt and Wagner, 2007). *Pelargonium sidoides* DC (*Geraniaceae*) is a herbaceous perennial with a long tradition of use in the treatment of gastrointestinal disorders, chest pain and bronchial infection among several ethnic groups in areas of Southern Africa, including Zulu, Bantu, Xhosa and Mfengu (Hutchings, 1996; Kolodziej and Kayser, 1998; Kolodziej, 2002). Following the well-documented therapeutic benefits against infections, an aqueous-ethanolic *P. sidoides* extract, EPs® 7630, has been elaborated from the traditional herbal medicine and successfully

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introduced in modern phytotherapy and is currently used to treat acute bronchitis (Brown, 2004; Conrad et al., 2007a, b). *In vitro* studies suggest that this extract has antimicrobial and immunomodulatory properties (Kayser and Kolodziej, 1997; Kolodziej et al., 2003). Since respiratory tract infections are frequently caused by viruses, the modulatory potential of this herbal medicine on the IFN system that may contribute to an improved antiviral protection is very important. Kolodziej and Kiderlen (2007) were able to demonstrate significant immunomodulatory properties of EPs® 7630. Antibacterial activity of extracts and isolated constituents of *P. sidoides* and *P. reniforme* (Andrews) Curtis has been evaluated previously (Kayser and Kolodziej, 1997). *Staphylococcus aureus*, *Streptococcus pneumoniae*, beta-hemolytic *Streptococcus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Haemophilus influenzae* were investigated by these authors. With the exception of the ineffective (+)-catechin, all the potentially active compounds exhibited antibacterial activities. Despite considerable efforts, bioactivity of *P. sidoides* can yet not be assigned to a chemically defined principle. EPs® 7630 contains a significant amount of proanthocyanidins that have beneficial effects on LPS-induced sickness behaviour, an effect which is under CNS control (Kolodziej, 2000; Nöldner and Schötz, 2007). Pretreatment of *Helicobacter pylori* with EPs® 7630 extract showed significant anti-adhesive activity against this bacterium (Wittschier et al., 2007). The bacterial adhesins, located on the outer cell wall, are responsible for interaction with mucosal glycoproteins and epithelial mucins and are blocked or inactivated by extract compounds. Conrad et al. (2007a, b) investigated phagocytosis, oxidative burst and intracellular killing of human peripheral blood phagocytes *in vitro* using *Candida albicans* as target organism. Intracellular killing of Candida was evaluated by determining the number of surviving yeast cells after co-incubation of the target organism and human whole blood. Compared with controls, EPs® 7630 increased the number of active peripheral blood phagocytes in a dose-dependent manner with a maximum augmentation of 120%. Intracellular killing of *Candida albicans* was also enhanced by this extract, revealing a biological activity of this extract. The clinical activity of EPs® 7630 has been mainly assigned to antiviral, antibacterial, immunomodulating and secretolytic properties.

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are agents of common infections with recurrent orofacial and genital lesions. HSV-1 predominantly causes epidermal lesions in and around the oral cavity. Genital herpes is a chronic, persistent infection mainly caused by HSV-2 spreading efficiently and silently as sexually transmitted disease through the population (Sucato et al., 1998). The hallmark of a herpes infection is the ability of the virus to establish a latent infection in the nervous system, to reactivate and to cause recrudescent lesions. The latent virus is reactivated spontaneously or is induced to reactivate by a variety of stimuli. During the reactivation process, the virus is transported through the nerve cells axons to the original peripheral infection site, where HSV replication occurs. Infectivity is highest in primary infections and virus excretion can persist for many weeks beyond clinical healing. Antiviral agents licensed currently for the treatment of herpesvirus infections include acyclovir and other inhibitors of the viral DNA polymerase, e.g. ganciclovir, foscarnet and cidofovir. Acyclovir, ganciclovir and cidofovir are nucleoside analogues which act as DNA chain terminators, ultimately preventing elongation of viral DNA. Foscarnet inhibits the viral DNA polymerase by binding to the pyrophosphate binding site. Acyclovir has been widely used for the management of herpes virus infections, its preferential phosphorylation by the HSV-encoded thymidine kinase makes it a selective antiviral drug (De Clercq, 2004). Some of these antiviral agents, e.g, ganciclovir and foscarnet, are associated with adverse effects. In addition, the emergence of virus strains resistant to commonly used anti-herpesvirus drugs is a growing problem, particularly in immunocompromised patients (Cassady and Whitley, 1997; Christophers et al., 1998). A large number of antiherpes screening experiments on medicinal plant extracts and plant-derived secondary metabolites (e.g. flavonoids, anthraquinones, naphthodianthrones, phenolics) have been reported (Reichling, 1999; De Logu et al., 2000). Antiherpes activity of several essential oils of different plant origin as well as of various essential oil constituents has been demonstrated (Sivropoulou et al., 1997; Benencia and Courrèges, 1999). Recently, anti-herpes activity of Australian tee tree oil (Schnitzler et al., 2001), manuka oil (Reichling et al., 2005), as well as antiviral activity of essential oils against clinical drug-resistant HSV-1 isolates (Schnitzler et al., 2007) have been reported. Several ethanolic and aqueous plant extracts from members of the family *Lamiaceae* have been shown to inhibit herpesvirus replication as well (Nolkemper et al., 2006).

However, antiviral properties of an aqueous *P. sidoides* root extract against herpesviruses have not been published. In the present study, the inhibitory activity of *Pelargonium sidoides* extract on HSV-1 and HSV-2 in cultured cells is reported. Furthermore, the mode of antiviral action of this extract at different steps of herpes virus replication is scrutinized.

Materials and methods

Plant material and reference substances

For preparing the aqueous extract, 100 ml of boiling water was added to 10 g of dried chopped roots of

Pelargonium sidoides, provided by Dr. Willmar Schwabe GmbH and Co. KG, Karlsruhe, Germany. Voucher specimens were deposited at the Dept. of Virology, University of Heidelberg. The resulting extract was filtered after 15 min and cooled down to room temperature, serially diluted with distilled water and added to the cell culture medium. To determine the dry weight, the extract was freeze-dried (Christ Alpha I-6 Heraeus, Hanau, Germany). For chemical characterization, 0.4 ml of this extract was dried in vacuum at a pressure of 10^{-3} mbar at room temperature (SpeedVac SPD 111 V, Savant, Düsseldorf, Germany). The obtained dry sample was re-dissolved in 2 ml aqueous methanol (80/20, MeOH/H_2O, v/v). As reference material, 4-hydroxy-coumarin (Merck, Darmstadt/Germany) was dissolved at known concentrations in aqueous methanol.

Chemical characterization of aqueous plant extract by HPLC and MS analyses

The HPLC system was a Merck-Hitachi LaChrom Elite (Merck, Darmstadt, Germany) consisting of a pump L-2130, an auto sampler L-2200, a JetStream column oven and a diode array detector L-2450. The column was a Sunfire C18 (250×4.6 mm i.d., 5 µm particle size; Waters, Wexford, Ireland) fitted with a security guard C18 ODS (4×3.0 mm i.d., Phenomenex, Torrance, USA) at a flow rate of 1 ml/min, a constant temperature of 25 °C. Eluent A was 5% aqueous formic acid, while B was 100% MeCN. Starting at 100% A for 5 min, a gradient was followed to 85% A at 60 min, 80% A at 75 min and 70% at 105 min, and 0% A at 110 min before re-equilibration to starting conditions. Based on a 7-point calibration quantification ($R^2 = 1$), the respective coumarin derivatives were quantified in duplicate (mean ± standard deviation) and expressed as 4-hydroxy-coumarin equivalents. Applying these chromatographic conditions, LC–MS analyses were performed on a an Agilent HPLC series 1100 (Agilent, Waldbronn, Germany) equipped with a degasser G1322A, a binary gradient pump G1312A, an auto sampler G1329/1330A, a column oven G1316A and a diode array detector G1315A connected in series with a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an ESI source operating in the positive ionization mode. Mass spectra were recorded in a range from m/z 50 to 700 at a target mass of m/z 200. Nitrogen was used as dry gas at a flow rate of 12 ml/min and a pressure of 70 psi. The nebulizer temperature was set to 365 °C. Using helium as collision gas (4.1×10^{-9} bar), collision-induced dissociation spectra were obtained with a fragmentation amplitude of 1.2 V (MS/MS).

Acyclovir

Acyclovir was purchased from GlaxoSmithKline (Bad Oldesloe, Germany) and dissolved in sterile water.

Cell culture and viruses

RC-37 cells (African green monkey kidney cells) were grown in monolayer culture with Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum (FCS), 100 µg/ml penicillin and 100 µg/ml streptomycin as described previously (Schuhmacher et al., 2003). Cells were plated out onto 24-well and 6-well culture plates for cytotoxicity and antiviral assays. Herpes simplex virus type 1 strain KOS and herpes simplex virus type 2 strain HG52 were routinely grown on RC-37 cells.

Cytotoxicity assay

Cells were seeded into 24-well plates and incubated for 24 h at 37 °C as described previously (Schnitzler et al., 2001). After 3 days of incubation with the drug, the growth medium was removed and viability of the drug-treated cells was determined in a standard neutral red assay. The cytotoxic concentration of the drug which reduced viable cell number by 50% (TC_{50}) was determined.

Plaque inhibition assay

Inhibition of virus replication was measured by a conventional plaque reduction assay. Serial dilutions of the extract-treated virus were adsorbed to RC-37 cells for 1 h at 37 °C. Subsequently the residual inoculum was discarded and infected cells were overlaid with medium containing 0.5% methylcellulose. After incubation for 3 days at 37 °C, monolayers were fixed with 10% formalin, stained with 1% crystal violet and subsequently the stained plaques were counted as described previously (Koch et al., 2008). The concentration of the drug which inhibited plaque numbers by 50% (IC_{50}) was determined from dose–response curves.

Mode of antiviral activity

Cells and viruses were incubated with acyclovir as positive control or aqueous *Pelargonium sidoides* extract at different stages during the viral infection cycle in order to determine the mode of antiviral action. Cells were pretreated with acyclovir or aqueous plant extract before viral infection, viruses were incubated with acyclovir or extract before infection and cells and viruses were incubated together with acyclovir or Pelargonium extract during adsorption or after penetration

of the virus into the host cells. The plant extract was always used at different concentrations up to the maximum noncytotoxic concentration. Cell monolayers were pretreated with the extract prior to inoculation with virus by adding the aqueous extract or acyclovir to the culture medium and by incubation for 1 h at 37 °C. The compound was aspirated and cells were washed immediately before the HSV-1 or HSV-2 inoculum was added. For pretreatment of herpes simplex virus type 1 and type 2, about 2×10^3 pfu of HSV were incubated in medium containing the maximum noncytotoxic concentration of the extract or acyclovir for 1 h at room temperature prior to infection of RC-37 cells. For analysing the antiviral inhibition during the adsorption period, herpesvirus was mixed with the drug or acyclovir and added to the cells immediately. After 1 h of adsorption at 37 °C, the inoculum was removed and cells were overlaid with medium containing 0.5% methylcellulose. The effect of Pelargonium extract and acyclovir against HSV was also tested during the replication period by adding the compounds after adsorption to the overlay medium, as typical performed in antiviral susceptibility studies.

Results

Chemical characterization of aqueous plant extract by HPLC and MS analyses

Three different compound classes in Pelargonium are known. Besides coumarins such as umckalin, scopoletin or fraxetin-derivatives, simple phenolic structures such as gallic acid and protocatechuic acids as well as flavonoid and catechin derivatives have been previously identified. The phenolic spectrum in *P. sidoides* was found to be quite complex, hence a gradient of 120 min was required to achieve reasonable compound separation (Fig. 1). Based on literature data, tentative assignments were possible, which however were complicated by the presence of isobaric coumarin derivatives. Since coumarin compounds are known to be the predominant constituents, 4-hydroxycoumarin was chosen as a representative compound for quantification purposes (Table 1). Due to oligomeric phenolic compounds (gallic acid derivatives and procyanidines) a high baseline resulted in HPLC, even after gradient optimisation. These oligomeric phenolic compounds were not removed prior to HPLC analysis in order to reflect the genuine coumarin spectrum. To the best of our knowledge, a HPLC separation of coumarin compounds from Pelargonium has not been reported previously.

Cytotoxicity and plaque inhibition assay

Monolayer cultures of RC-37 cells were grown in 0.000001–10% drug-containing medium and after 3 days of incubation, cell viability was determined in the neutral red assay. The toxic concentration (TC_{50}) of *P. sidoides* extract for RC-37 cells was determined at 0.04% (Fig. 2). The potential inhibitory effect of *P. sidoides* extract against herpesviruses was determined by pretreatment of viruses with the extract for 1 h at room temperature and subsequent infection of RC-37 cells. IC_{50} for *P. sidoides* extract was determined from

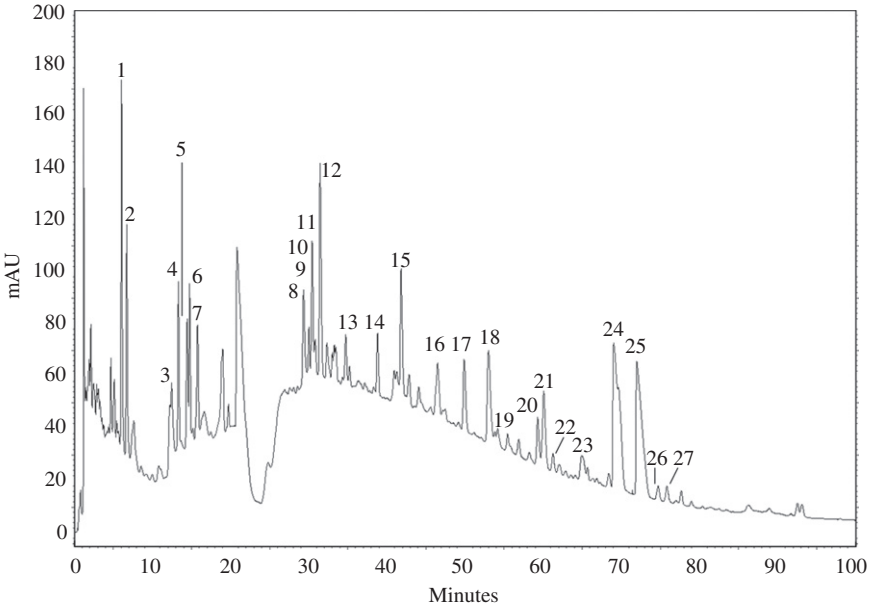

Fig. 1. HPLC chromatogram of an aqueous *Pelargonium sidoides* extract at 260 nm.

Table 1. Characteristic data of selected compounds from *P. sidoides* aqueous extract and their quantification as 4-hydroxycoumarin equivalents

Peak-no.	R_t (min)	λ_{max} (nm)	m/z $(M+H)^+$	MS^2 m/z $(M+H)^+$	MS^3 m/z $(M+H)^+$	4-hydroxycoumarin equivalents (mg/l)	Assignment
1	8.0	245, 272	300	282	264, 241, 150	14.21 ± 0.08	–[a]
2	8.7	246, 257sh, 300sh	241	223	164	9.62 ± 0.05	–[a]
3	14.1	244, 273, 306	355	171	153	1.81 ± 0.34	Glucogallin
4	16.2	245, 264sh, 304	330	312	204, 136	5.65 ± 0.01	–[a]
5	17.1	245, 270, 301sh	307	289	139	3.17 ± 1.64	Phyllanthusiin E-methylester[b]
6	18.2	245, 270	346	152	135	3.93 ± 0,52	–[a]
7	18.8	245, 265sh, 305	305	277	259, 151	5.56 ± 0,06	–[a]
8a	32.3	246, 284, 301, 341	275	195	149	4.17 ± 1.62	Dihydroxy-coumarin-sulfate or 6,8-Dihydroxy-5,7-dimethoxycoumarin (Na^+)
8b	32.5	246, 284, 301, 341	371	209	149		Fraxetin-7-O-glucoside [M^+]; 209: Fraxetin
8c	32.6	246, 284, 301, 341	318 [M^+]		301, 265, 239, 205, 175		Monohydroxy-dimethoxy-coumarin-sulfat[c]
9	33.3	245, 275, 300sh	357	195	149	1.30 ± 0.50	4-Allyl-2,5-dimethoxyphenol-1-glucose
10	33.9	246, 270, 305sh	307	289, 139	139	5.70 ± 0.49	Phyllantusiin-E-methylester[b]
11	34.4	245, 278, 306sh	291	273, 209, 123	123	1.21 ± 0.30	Catechin
12	34.8	246, 259sh, 280, 318	275	195	149	12.99 ± 0.09	Dihydroxy-coumarin-sulfate
13	38.5	245, 268, 297sh, 334sh	341	179	151	2.46 ± 0.10	Dihydroxy-coumarin-hexoside[c]
14	43.8	245, 271, 301	497	331	312, 219, 187	3.19 ± 0.06	–[a]
15	46.7	245, 254sh, 287sh, 300	289	209	194, 149, 121	8.02 ± 0.69	Fraxetinsulfate or Isofraxetinsulfate
16	51.7	246, 275, 300sh, 335	223	208	188	5.03 ± 0.05	Monohydroxy-dimethoxycoumarin
17	55.0	246, 280, 336	273	255, 193	133	5.66 ± 0.04	–[a]
18a	58.2	245, 289, 336sh	385	349, 287, 259, 223, 153	231, 149	8.96 ± 0.18	–[a]
18b	58.5	245, 289, 336sh	305	287, 259	231, 149		Taxifolin[b]
19	59.9	247, 277, 299sh	253	238	220, 192	0.93 ± 0.03	Dihydroxy-dimethoxycoumarin
20	64.8	247, 291, 336sh	305	153	123, 68	2.99 ± 0.22	Taxifolin[b]
21	66.0	248, 276	186	163	163	6.33 ± 0.27	–[a]
22	66.9		239	224	206	1.29 ± 0.01	Dihydroxy-dimethoxycoumarin
23	70.6	248, 293	289	243	215, 149	1.58 ± 1.02	Dihydrokaempferol[b]
24	74.8	248, 256sh, 316	319	239	224	10.03 ± 0.74	Monohydroxy-dimethoxy-coumarin-sulfate
25	77.7	248, 298, 331sh	223	208	163	25.74 ± 006	Mono-hydroxy-dimethoxy-coumarin (most probably Umckalin = 7-Hydroxy-5,6-dimethoxycumarin)
26	80.2	249, 291	319	301	273, 195, 153	1.24 ± 0.01	–[a]
27	81.4	250, 284sh, 326	223	208	179	1.36 ± 007	Monohydroxy-dimethoxy-coumarin

[a]No assignment possible.
[b]Tentative assignment.
[c]Assignment of the respective isomer not achieved.

dose–response curves at 0.00006% and 0.000005% for HSV-1 and HSV-2, respectively, and a dose-dependent activity of the extract could be demonstrated.

Mode of antiviral activity

To identify the step at which replication might be inhibited, cells were infected with HSV-1 or HSV-2 after preincubation of the cells with acyclovir as positive control or *P. sidoides* extract, pretreatment of the virus with acyclovir or the plant extract prior to infection, addition of the synthetic antiviral drug or aqueous extract during adsorption or after adsorption during the intracellular replication period. In all experiments cells infected with untreated virus were used as control. The percent reduction was calculated relative to the amount of virus produced in the absence of the drug. Acyclovir showed the maximum antiviral activity when added at a concentration of 22.5 µg/ml during the replication period with inhibition of the viral replication of more than 98% for both herpesviruses (Fig. 3). This drug inhibits specifically the viral DNA polymerase during the replication cycle when new viral DNA is synthesized. However, no significant effect on viral replication was detected when acyclovir was used for pretreatment of cells or viruses or when acyclovir was only added during the adsorption phase. In all antiviral plant extract assays different extract concentrations up to the maximum noncytotoxic concentration were used and the results are shown in Fig. 4 (HSV-1) and Fig. 5 (HSV-2). Pretreatment of cells with the extract and addition of the drug during the replication phase did not reduce virus production. However, pretreatment of HSV with the analysed plant extract prior to infection and addition of the aqueous extract during the adsorption phase of HSV

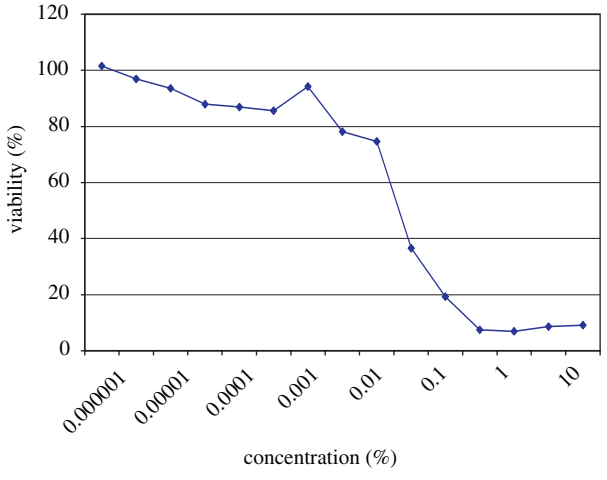

Fig. 2. Cytotoxicity of *Pelargonium sidoides* extract on RC-37 cells as determined with the neutral red assay. Cells were seeded in 24-well microtiter plates, medium was removed and the cells were incubated with neutral red for 3 h. Cell viability was determined at 540 nm and optical density was compared with the uptake of the dye to untreated controls. The optical densities of drug-treated cells are expressed as a percentage of control cells. The values are the average of four independent experiments.

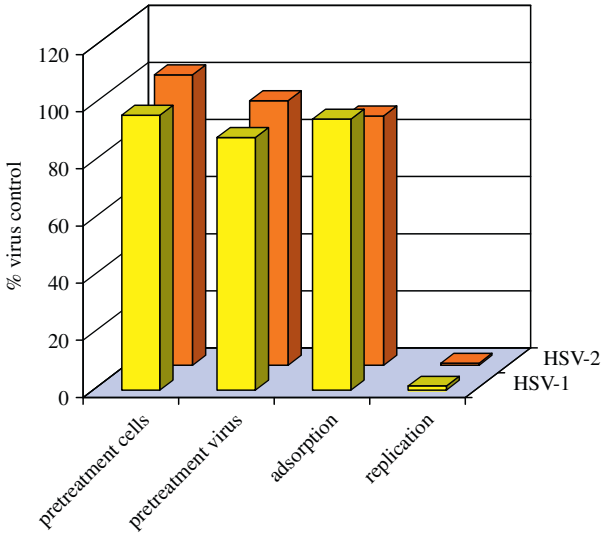

Fig. 3. Antiviral effect of the antiviral drug acyclovir against HSV-1 and HSV-2 by incubation at different periods of time during infection. Cells were pretreated with acyclovir prior to virus infection (pretreatment cells), viruses were pretreated prior to infection (pretreatment virus), the approved antiviral drug was added during the adsorption period (adsorption) or after penetration of the viruses into cells (replication). Experiments were repeated independently three times and data presented are the average of four experiments.

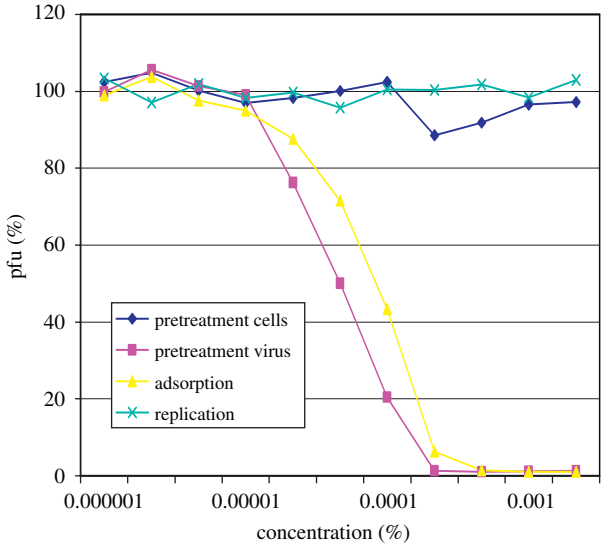

Fig. 4. Antiviral effect of *Pelargonium sidoides* extract against HSV-1, details see Fig. 3. Dose–response experiments were repeated independently three times and data presented are the average of four experiments.

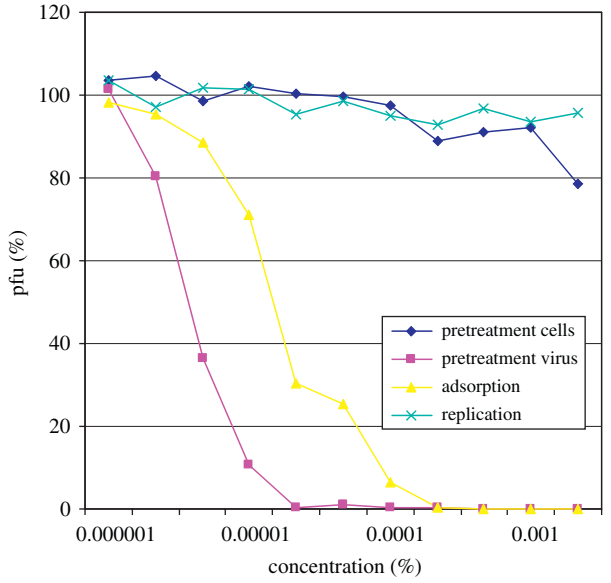

Fig. 5. Antiviral effect of *Pelargonium sidoides* extract against HSV-2, details see Fig. 3. Data presented are the mean of four experiments.

caused a rapid and significant reduction of infectivity. HSV-1 titer was reduced by more than 99% at a concentration of 0.0003% *P. sidoides* extract, whereas HSV-2 was reduced by more than 99% at a lower extract concentration of 0.00003%. These results indicate that the antiviral effect of the investigated essential oils is mainly exerted prior to adsorption of HSV to the host cells.

Discussion

Therapeutic benefits against infections have been reported for an aqueous-ethanolic extract of the traditional South African herb *Pelargonium sidoides*. This extract reveals antibacterial activities (Kayser et al., 2001; Neugebauer et al., 2005) and has been successfully introduced in modern phytotherapy and is currently used to treat acute bronchitis (Brown, 2004; Matthys et al., 2007). The potential antiviral effect of an aqueous *P. sidoides* extract was determined against HSV-1 and HSV-2 *in vitro*. Herpes simplex virus type 1 is transmitted through contact with saliva and causes recurrent herpes labialis, whereas HSV-2 is transmitted primarily sexually and is the causative agent of genital herpes. Main constituents of the aqueous extract were identified by HPLC–MS analyses and comprised coumarins, simple phenolic structures as well as flavonoid and catechin derivatives being in accordance with literature data (Kayser and Kolodziej, 1997; Latté et al., 2000; Gödecke et al., 2005). Among the compounds previously reported to constitute the phenolic patterns of *Pelargonium sidoides* and *P. reniforme*, coumarin derivatives were found to be predominant in the present study. Therefore, 4-hydroxycoumarin was chosen as a representative compound for quantitative measurements. Compared to published data (Kayser and Kolodziej, 1997; Latté et al., 2000; Gödecke et al., 2005), qualitative and quantitative differences may be explained by varying starting material, but also different extraction conditions. Since literature data on isomeric coumarin derivatives were not available, thorough assignment even after mass spectrometric investigations was not possible. Latté et al. (2000) reported tri- and tetra-oxygenated coumarins, gallic acid, gallic acid methyl esters and various flavonoids to contribute to the bioactive constituents of umckaloabo and described two distinct coumarins, umckalin and three unique coumarin sulphates. Since *P. reniforme* was reported to exhibit lowest flavonoid contents in root, followed by flowers and highest in herbs (Latté et al., 2000), the current findings for *P. sidoides* with low flavonoid contents appear to be plausible. Bacterial kinetic assays using coumarins of Pelargonium demonstrated the antibacterial activity to be bacteriostatic (Kayser and Kolodziej, 1997). Schötz and Nöldner (2007) fractionated proanthocyanidin oligomers from an aqueous-ethanolic *P. sidoides* extract. *In vivo* studies with these fractions investigating motility, body weight gain, body temperature, motoric coordination, anticonvulsant effects and central analgesic activities showed no or only moderate pharmacological effects.

Experiments to assess the toxicity of Pelargonium extract for cultured eucaryotic cells indicate a moderate toxic behaviour in cell cultures. The toxicity of aqueous *P. sidoides* extract approached 50% (TC_{50}) at an extract concentration of 0.04%. In plaque reduction assays the plant extract exhibited a concentration-dependent antiviral effect, when HSV was mixed with the extract prior to inoculation. Using maximal noncytotoxic concentrations of this plant extract in viral suspension assays, plaque formation was lowered by about 99.9% for HSV-1 and HSV-2. Similar effects against herpesvirus have been reported for several plant-derived essential oils (Schuhmacher et al., 2003). Interestingly, the extract was quite more effective against HSV-2, and inactivated the virus at a tenfold lower concentration as compared to HSV-1. In order to determine the mode of antiviral action, either cells were pretreated before viral infection or viruses were incubated with noncytotoxic concentration of acyclovir or *P. sidoides* extract before infection, during adsorption or after penetration into the host cells. Acyclovir reduced plaque formation most when added during the replication period when this nucleoside analogon is incorporated into viral DNA. Pretreatment of the cells with the Pelargonium extract had no effect on the production of infectious virus and plaque formation was not affected. However, pretreatment of

HSV-1 and HSV-2 with this plant extract prior to infection or addition of the extract during the adsorption phase resulted in a dose-dependent reduction of plaques, suggesting that *P. sidoides* extract interferes with virion envelope structures or is masking viral compounds which are necessary for adsorption or entry into host cells. The obvious anti-HSV effect of *P. sidoides* extract might be due to the presence of oligomeric phenolic compounds like proanthocyanidines and derivatives of gallic acids (Reichling, 1999). These results allow to presume that free virus is very sensitive to the antiviral compounds of the aqueous extract, the inhibition of HSV appears to occur before entering the cell but not after penetration of the virus into the cell. It remains to be determined whether the inhibitory effect is due to binding of some constituents of the extract to viral proteins involved in host cell adsorption and penetration or is due to damage of the virions, possibly their envelopes, thereby impairing their ability to infect host cells.

The application of plant-derived products against viral and bacterial infections has been described previously (Reichling, 2001). Tea tree oil, the essential oil of *Melaleuca alternifolia* Cheel, has been applied successfully in the treatment of recurrent herpes labialis (Carson et al., 2001) as well as for methicillin-resistant *Staphylococcus aureus* (MRSA) on the skin of infected patients. The topical use of the antiviral aqueous *P. sidoides* extract for the treatment of herpetic infections appears promising, especially for those patients suffering from frequent recurrences.

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