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Efficacy of an aqueous *Pelargonium sidoides* extract against herpesvirus

P. Schnitzler[a,*], S. Schneider[a], F.C. Stintzing[b], R. Carle[b], J. Reichling[c]

[a]*Department of Virology, Hygiene Institute, University of Heidelberg, 69120 Heidelberg, Germany*
[b]*Institute of Food Sciences and Biotechnology, Hohenheim University, Stuttgart, Germany*
[c]*Department of Biology, Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, Heidelberg, Germany*

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

*Corresponding author. Tel.: +49 6221 565016; fax: +49 6221 565003.
E-mail address: Paul_Schnitzler@med.uni-heidelberg.de (P. Schnitzler).

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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