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The antiviral activity of six South African plants traditionally used against infections in ethnoveterinary medicine

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ABSTRACT

Viral infections remain a major threat to humans and animals and there is a crucial need for new antiviral agents especially with the development of resistant viruses. The hexane, dichloromethane, acetone and methanol extracts of six plant species selected for their traditional use against infections were tested for *in vitro* antiviral activity against canine distemper virus (CDV), canine parainfluenza virus-2 (CPIV-2), feline herpesvirus-1 (FHV-1) and lumpy skin disease virus (LSDV). All extracts were tested for their cytotoxicity using a colorimetric tetrazolium-based (MTT) assay and were tested for antiviral efficacy at concentrations below CC_{50} values on the various cell types used in this study. The antiviral activity of extracts was tested using virucidal and attachment assays. In the virucidal assay, extracts were incubated with virus prior to infection. The most potent inhibition was observed with the acetone and methanol extracts of *Podocarpus henkelii* against CDV and LSDV, which inhibited replication of the viruses by >75% at 3 μg/ml with selectivity index (SI) values ranging between 12 and 45. Excellent activity was also found with the hexane extracts of *Plumbago zeylanica* and *Carissa edulis* against CDV, with the extracts reducing viral-induced CPE by 50% and 75% respectively. The hexane extract of *C. edulis* had moderate activity against FHV-1 with $EC_{50} < 70$ μg/ml and SI value <2. Only the acetone extract of *P. henkelii* moderately inhibited replication of LSD virus in the attachment assay, with low activity in other extracts. Of the four extracts with significant antiviral activity, two were prepared from *P. henkelii*. Therefore, future work will focus on isolating and characterizing the substance(s) responsible for bioactivity in extracts of this species.

1. Introduction

Unlike bacterial cells, which are free-living entities, viruses are obligate intracellular parasites, which contain little more than bundles of gene strands of either RNA or DNA, and may be surrounded by a lipid-containing envelope (Wagner and Hewlett, 1999). Every strain of virus has its own unique arrangement of surface molecules, which aids in its attachment to host cells. Following attachment, viruses utilise the host cell they invade to propagate new viruses. Hence their successes in nature have been attributed to differences in their genetic compositions, means of transmission, efficient replication within host cells, and their ability to persist in the host (Wagner and Hewlett, 1999). As such they cause various diseases in humans, animals and plants alike. Most alarming is the lack of effective treatment for many viral infections coupled with selection of resistant and cross-resistant mutants as well as the potential toxic effect of presently available therapeutics.

The use of herbal remedies has gained increased recognition globally within the last decades. The World

Abbreviations: CDV, canine distemper virus; LSDV, lumpy skin disease virus; CPIV-2, canine parainfluenza virus-2; FHV-1, feline herpesvirus-1.

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Health Organisation (WHO) in 2001 reported that about 80% of the world's population, especially those people in developing countries, rely on medicinal plants to treat various ailments. About 20,000 of the plant species used for these purposes have been documented by WHO (Gullece et al., 2006). Medicinal plants with strong antiviral activity to treat viral infections in humans and animals have been identified and those containing novel plant-derived antiviral agents with complementary and overlapping mechanisms of action have been studied (Venkateswaran et al., 1987; Hudson, 1990; Thyagarajan et al., 1990; Chattopadhyay and Naik, 2007). Medicinal plants are progressively being explored as appropriate alternative sources for discovery of antiviral agents (Williams, 2001; Jassim and Naji, 2003; Camargo Filho et al., 2008; Lupini et al., 2009; Choi et al., 2009) and more research is ongoing. Natural products, either as standardised plant extracts or pure compounds, comprise substances with diverse chemical structures, providing an unlimited pool of new drug leads (Vlietinck et al., 2006), possibly with less toxic effects. Additionally, studies evidencing the antiviral potential of plant extracts against viral strains resistant to conventional antiviral agents (Serkedjieva, 2003; Tolo et al., 2006) highlight the need for exploring medicinal plants for natural antiviral components.

Feline herpesvirus-1 (FHV-1) is the most common viral pathogen of domestic cats worldwide. It causes infections of the eye characterised by conjunctivitis, and profuse ocular and nasal discharges. In severe cases, disease progression leads to keratitis and ulceration of the cornea as well as severe upper respiratory tract involvement (Gaskell and Dawson, 1994; Gaskell and Willoughby, 1999; Andrew, 2001; Maggs, 2005). In contrast, canine distemper virus (CDV) infection affects predominantly canines, which serve as the natural host of the virus (Deem et al., 2000). The virus causes highly contagious, systemic disease in dogs world-wide. Despite the fact that infection of dogs may result in an array of clinical forms, immunosuppression and demyelinating leukoencephalitis characterise the main outcome in this species (Krakowka et al., 1985; Appel, 1987). Dogs naturally infected with CDV may also serve as alternative animal models to study the pathogenesis of demyelinisation in various diseases, including multiple sclerosis (Baumgärtner and Alldinger, 2005; Vandevelde and Zurbriggen, 2005; Beineke et al., 2009). Canine parainfluenza virus-2 (CPIV-2) is another pathogen that affects dogs. It is closely related to simian virus 5 (SV5), human SV5 related isolates, porcine, ovine and feline parainfluenza viruses and to a lesser extent, the mumps virus (Randall et al., 1987; Ajiki et al., 1982). The virus is one of several pathogens that cause kennel cough in dogs. Natural infection with CPIV-2 in dogs is self-limiting and restricted to the upper respiratory tract although some authors have reported the isolation of the virus from organs other than the respiratory tract (Evermann et al., 1980; Macartney et al., 1985).

Lumpy skin disease virus (LSDV) affects cattle and is caused by a capripox virus. The disease is infectious, eruptive and occasionally fatal, affecting cattle of all ages and breeds. It is characterised by fever, skin nodules, necrotic plaques in mucosae and lymphadenopathy. During outbreaks, morbidity may be as high as 100% and mortality up to 40%. Severe economic losses during outbreaks are associated with emaciation, damage to hides, infertility in males and females, mastitis and reduced milk production (Barnard et al., 1994).

Viral infections can be controlled either through prophylactic or therapeutic intervention. Although vaccines are available to control these infections, no effective antiviral therapy for the treatment of these diseases currently exists. The availability of vaccines to the majority of the people living in rural areas, particularly in developing countries, is limited owing to economic constraints. Therapeutic inhibition of virus infection may well involve a number of strategies and may target various steps in the life cycle of the virus such as cell entry, virus replication, or the assembly and release of virions. Four enveloped viruses were selected for this study based on the assumption that targeting the entry of enveloped viruses may be a strategic approach for therapeutic intervention, given that the site of action of the substances that will inhibit the virus is likely to be extracellular and reasonably accessible by the inhibitor. As representative viral targets, two DNA- and two RNA-containing viruses of veterinary importance in South Africa were included in this study. The overall objective of the present research was to assess the antiviral effect against canine distemper virus (CDV), canine parainfluenza virus-2 (CPIV-2), lumpy skin disease virus (LSDV) and feline herpesvirus-1 (FHV-1) of different extracts of selected medicinal plants with ethnobotanical uses in South African folk medicine. The plants were selected on the basis of their traditional indications in treating various infections in humans and animals.

2. Materials and methods

2.1. Plant collection and preparation of extracts

Leaves of six plants were collected from the Lowveld National Botanical Garden (NBG) in Nelspruit, Mpumalanga province, South Africa, in the month of April. Voucher specimens are present in the herbarium at the Lowveld NBG, or in the National Herbarium in the Pretoria National Botanical Garden (Table 1). The leaf material was air-dried at room temperature and milled into fine powder using a Macsalab mill (Model 200 LAB) Eriseo, Bramley. The traditional indications of the plants selected for the studies are represented in Table 1. Separate aliquots of ground plant material were extracted (10:1 solvent to dry plant material) using hexane, dichloromethane, acetone and methanol. Each extract was dried under low temperature before being reconstituted to 100 mg/ml in DMSO. Serial 10-fold dilutions in cell culture medium were prepared to final concentrations of 1, 0.1, 0.01, and 0.001 mg/ml to determine their cytotoxic concentrations.

2.2. Virus culture

The following viruses were used in this study: feline herpesvirus-1 (FHV-1, dsDNA), canine distemper virus (CDV, ssRNA), canine parainfluenza virus-2 (CPIV-2, ssRNA)